

Third Edition

Genetics and the Behavior of Domestic Animals

Edited by

Temple Grandin



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Edited by

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Preface

The first edition of *Genetics and the Behavior of Domestic Animals* was published almost 25 years ago. This third edition contains both the latest research and older studies that will provide valuable insights for both scientists and people who work hands-on with farm animals. It is important for students and other people entering the field of behavioral genetics to be exposed to some of the older observational work. New genomic technologies are now starting to validate many of these earlier observations (Johnson et al., 2018). Both scientists and people who work directly with farm animals should never forget that good animal behavior research is based on careful observations of behavior. These observations are then used to form a hypothesis for new research. An understanding of emotions in animals will serve as an important conceptual framework for determining how genes influence animal behavior. Behavioral traits in animals are often influenced by multiple genes (Johnson et al., 2018).

Scientific thinking on the existence of animal emotions has completely changed since the publication of the first edition. Over the last 30 years, the science of emotions in animals evolved from ignoring the emotional lives of animals to validating that they have a variety of emotional experiences (DeWaal, 2011). In 1998 when the first edition was published, the existence of one emotion, fear, was recognized in domestic farm animals. At this time, Alain Boissey in France, Paul Hemsworth from Australia, and I had already started using the word fear in scientific publications about farm animals (Boissey, 1995; Hemsworth et al., 1990; Grandin, 1997). Before this time, journal article reviewers forced me to remove the word fear from a study of the behavior of cattle during handling and replace it with agitated behavior (Grandin, 1993). Both the early ethologists who studied the behavior of animals in their natural environment and the behaviorists who studied behavior in the laboratory avoided the word emotion. The famous behaviorist B.F. Skinner stated that animals did not have inner emotional lives (Skinner, 1977). Both disciplines recognized that genetic factors had an effect on behavior but emotions were omitted. Jaak Panksepp's views on seven basic subcortical emotional systems became widely known when he published his book "Affective Neuroscience" (Panksepp, 1998). They are FEAR, RAGE, PANIC, SEEK, LUST, CARE, and PLAY. Panksepp fully explains his work

in two papers (Panksepp, 2011; Panksepp et al., 2017). There is an outline of his work in the first chapter of this book. His basic premise is that emotional systems that influence behavior are driven by subcortical sites in the brain. The brain's higher thinking centers in the cortex are not required to experience emotions. Direct stimulation of subcortical sites will trigger an emotional response, such as rage in cats (Hess, 1957). Hess originally called the cat's reaction "sham" rage because he feared being marginalized by the behaviorists (Panksepp, 2011). Juvenile rats without a cortex will engage in play (Panksepp et al., 1994). If the cortex is removed at birth when the cat becomes an adult, it will have normal sexual behavior and nurse its kittens (Bjursten et al., 1976). This illustrates that the LUST and NURTURE systems were still intact. Further evidence that emotions originate from lower brain centers is research with children who are born without a cortex. They clearly can experience positive emotions and they will smile when they are with their favorite people (Shewman et al., 2007). For many years, some researchers ignored Panksepp's work. Cutting edge research published since the second edition clearly shows that emotions in both people and animals are influenced by the same genetic mechanisms (Persson et al., 2016). In dogs, the friendliness trait is influenced by the same genes that are associated with William's syndrome in humans (Von Holdt et al., 2017). A William's child will be very friendly and have intellectual deficits. In cattle, behavior during handing has been assessed in countless studies. They are reviewed in the fourth chapter of this edition and in Grandin (2019). The genetic factors that influence behavior in cattle are also associated with autism (Costella et al., 2020).

Reser (2014) has an interesting paper titled "Solitary Mammals Provide an Animal Model for Autism Spectrum Disorders." Solitary mammals, such as a panther or a leopard, have both hormonal and genetic factors associated with autism when they are compared to more social mammals. Why would human disorders in humans be associated with normal behavioral variations in the emotional behavior of animals? Maybe it is the price humans must pay for a more complex and larger brain. When a brain becomes more complex, it may be more likely to have wiring abnormalities. In the second edition from 2014, studies on positive emotions were included in a chapter by Alain Boissey and Hans Erhard. This same chapter has been reprinted in this new edition.

Since the second edition published in 2014, the word "personality" now appears in research studies of farm animals (Wilson et al., 2019; O'Malley et al., 2018). Fear is not the only emotional system that is being studied. In animal personality research, fear is described as bold versus shy. The terms high and low exploration would be the SEEK trait, and differences in aggressiveness would be differences in the RAGE trait. It will be interesting to

examine a classic old study by Jean Michael Faure and Andrew D. Mills using the latest genomic tools. This study that originally appeared in the first edition has been reproduced in this third edition under a new title of "Genetic Selection of Poultry for Behavioural Traits to Improve Welfare." It was one of the first studies to use conventional breeding methods to select birds for two separate behavioral traits of fearfulness and social reinstatement. This is the type of study that should be carefully studied with modern genomics. Social reinstatement is an awkward academic term for the motivation of an isolated animal to rejoining other animals. It is a behavioral trait that is distinct from fear. It is possibly related to Panksepp's PANIC (separation and anxiety) trait. Many genetic factors influence the social reinstatement trait (Johnson et al., 2018).

It is interesting that a book about genetics and domestic animal behavior has been widely read by researchers studying wildlife (McDougal et al., 2005; May et al., 2016). A literature search showed many wildlife-related citations. Some wildlife specialists are concerned that wild animals living in environments managed by humans may start showing traits of domestic animals. Behaviors that help an animal survive in the wild, such as high fear, are detrimental in captive environments (May et al., 2016). In science, it is important for different disciplines to communicate, and as an editor of this book, I am pleased that researchers outside the field of domestic animals have found this book useful.

In this third edition, the chapters on cattle, pigs, and horse behavior have all been completely updated. Two new chapters on genetic influences of dog behavior have been added, along with an updated chapter on genetic effects on animal welfare. Since the second edition was published in 2014, there has been an increased emphasis on the importance of farm animals having positive emotions. Preventing fear and suffering is not sufficient (Mellor et al., 2020). The farm animal should have a life worth living.

To provide a worldwide perspective, this edition features chapters written by authors from Australia, Central America, France, Russia, Sweden, and the United States. This book will be of interest to students in animal behavior, biologists, veterinarians, zoo managers, researchers, evolutionary biologists, and animal welfare specialists. People who own or train domestic animals will also find this book useful. To make the book accessible to people outside the field of genetics, the use of jargon and long technical explanations of genetics have been avoided.

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Chapter 1

Behavioral genetics and animal science

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ABSTRACT
The partnership between humans and domestic animals is natural. The human brain is hard-wired to emotionally respond to animals. Beginning with the domestication of wolves, this chapter covers the process of domestication and reviews the early work of behaviorists and ethologists who refused to accept emotional states in animals. Modern behavior research employs methods developed by behaviorists and ethologists combined with neuroscience and genetics. Emotional systems in the brain drive behavior. Confusion between different emotional systems may explain conflicting findings in the behavior literature. Behavior in an open-field test may be motivated either by fear, separation distress, or novelty seeking. Each emotion is controlled by separate subcorticol systems. A novel open-field arena can frighten a prey species, but it may activate seeking in a predator. Genetics affects the strength of fear, novelty seeking, and separation distress. Behavior is shaped by a complex interaction between genetics and experience.

Introduction

There have been huge advances in genetics since the publication of the first edition in 1998 and the second edition in 2014. This chapter contains many older studies that still provide valuable insights into genetic effects on animal behavior. It has been fully updated with information from the latest research. The original introduction from the first edition has been kept.

A bright orange sun is setting on a prehistoric horizon. A lone hunter is on his way home from a bad day at hunting. As he crosses the last ridge before home, a quick movement in the rocks, off to his right, catches his attention. Investigating, he discovers some wolf pups hiding in a shallow den. He exclaims, "Wow . . . cool! The predator . . . in infant form."

After a quick scan of the area for adult wolves, he cautiously approaches. The pups are all clearly frightened and huddle close together as he kneels in

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front of the den . . . all except one. The darkest-colored pup shows no fear of the man's approach. "Come here you little predator! Let me take a look at you," he says. After a mutual bout of petting by the man and licking by the wolf, the man suddenly has an idea. "If I take you home with me tonight, maybe mom and the kids will forgive me for not catching dinner . . . again."

The opening paragraphs depict a hypothetical scenario of a man first taming the wolf. Although we have tried to make light of this event, the fact is no one knows exactly how or why this first encounter took place. More than likely, the "first" encounter between people and wolves occurred more than once. New technologies that have been recently developed enable the extraction of DNA from ancient fossils (Frantz et al., 2020; McHugo et al., 2019). The earliest possible dates for domestication may have been 25,000−40,000 years ago (Pavlidis and Somel, 2020). Previous studies suggest that dogs were domesticated 14,000 years ago (Boessneck, 1985). However, Ovodov et al. (2011) reported finding dog fossils 33,000 years old in Siberia. Domestication of dogs may have begun before 35,000 years ago in what Galibert et al. (2011) described as a period of proto-domestication. Early hunter−gatherers may have captured wolf pups that became tame and habituated to living with human groups. Some wolves may have become aggressive as they matured and were killed or chased away. Others remained submissive and bred with less fearful wolves scavenging around human settlements. Analysis of mitochondrial DNA of 67 dog breeds and wolves from 27 localities indicates that dogs may have diverged from wolves over 100,000 years ago (Vita et al., 1997). Other researchers question this finding and suggest that dogs were domesticated 5400−16,300 years ago from many maternal lines (Pang et al., 2009; Savolainen et al., 2002). Other evidence suggests that domestication occurred in several different regions (Boyko, 2011). Ancient breeds such as the Australian Dingo, Basenjis, and New Guinea singing dogs, all originated in areas where there were no wolves (Larsen et al., 2012). Possibly, the scenario at the beginning of this chapter happened many times.

Another scenario is that wolves domesticated themselves. The presumption is that calm wolves with low levels of fear were more likely to scavenge near human settlements. Both Coppinger and Smith (1983) and Zenner (1963) suggest that wild species that later became domesticants started out as camp followers. Some wolves were believed to have scavenged near human settlements or followed hunting parties. Modern dog breeds probably were selectively bred from feral village dogs (Boyko, 2011).

The human brain is biologically programmed to pay attention to animals. Electrical recordings from the human amygdala, a brain structure involved with emotion, showed that pictures of animals caused a larger response than pictures of landmarks, people, or objects (Morman et al., 2011). Both threatening and nonthreatening animal pictures evoked the same response. Maybe this shows the importance of animals in our past.

Genetics shapes behavior

Genetic differences in animals affect behavior. A major goal of the third edition of this book is to review both old and new research on individual differences within a breed and behavioral differences between breeds. Well-done behavioral studies never become obsolete. Scientists may discover new ways to interpret "why" a certain behavior occurs but well-done behavioral studies always retain their value. During our literature review, we found many new studies that verified older studies reviewed in the first edition. A good example is a classic work on fearfulness and social reinstatement (separation distress) in quail (Faure and Mills, 1998, Chapter 11). These emotional traits can be independently strengthened or weakened by selective breeding. Recent experiments with foxes showed distinct differences in the genome of foxes bred to be either tame or aggressive (Kukekova et al., 2018).

This book is aimed at students, animal breeders, researchers, and anyone who is interested in animal behavior. There has been a great increase in research on genetic mechanisms that affect both behavior and physical traits of animals. In this chapter, we review studies of behavioral differences with a focus on domestic animals such as dogs, cattle, horses, pigs, sheep, and poultry. Detailed reviews of genetic mechanisms and molecular biology are beyond the scope of this book.

Genetic effects of domestication

Price (1984) defined domestication as follows:

> *a process by which a population of animals becomes adapted to man and the captive environment by some combination of genetic changes occurring over generations and environmentally induced developmental events recurring during each generation.*

Major behavioral differences exist between domesticated animals and their wild relatives. For example, the jungle fowl is much more fearful of novel objects and strange people compared to the domestic white Leghorn chicken (Campler et al., 2008). A strong genetic component underlies differences in fearfulness between jungle fowl and domestic chickens (Agnvall et al., 2012).

Domestication may have been based on selection for tameness. In long-term selection experiments designed to study the consequences of selection for the "tame" domesticated type of behavior, Belyaev (1979) and Belyaev et al. (1981) studied foxes reared for their fur. The red fox (*Vulpes fulva*) has been raised on seminatural fur farms for over 100 years and was selected for fur traits and not behavioral traits. However, the foxes had three distinctly different behavioral responses to people. Thirty percent were extremely

aggressive, 60% were either fearful or fearfully aggressive and 10% displayed a quiet exploratory reaction without either fear or aggression. The objective of this experiment was to breed animals similar in behavior to domestic dogs. By selecting and breeding the tamest individuals, 20 years later the experiment succeeded in turning wild foxes into tame, border collie-like fox-dogs. The highly selected "tame" population of (fox-dog) foxes actively sought human contact and would whine and wag their tails when people approached (Belyaev, 1979). This behavior was in sharp contrast to wild foxes that showed extremely aggressive and fearful behavior toward men. Keeler et al. (1970) described this behavior:

Vulpes fulva (the wild fox) is a bundle of jangled nerves. We had observed that when first brought into captivity as an adult, the red fox displays a number of symptoms that are in many ways similar to those observed in psychosis. They resemble a wide variety of phobias, especially fear of open spaces, movement, white objects, sounds, eyes or lenses, large objects, and man, and they exhibit panic, anxiety, fear, apprehension, and a deep trust in the environment. They are (1) catalepsy-like frozen positions, accompanied by blank stares, (2) fear of sitting down, (3) withdrawal, (4) runaway flight reactions, and (5) aggressiveness. Sometimes the strain of captivity makes them deeply disturbed and confused or may produce a depression-like state. Extreme excitation and restlessness may also be observed in some individuals in response to many changes in the physical environment. Most adult red foxes soon after capture break off their canine teeth on the mesh of our expanded metal cage in their attempts to escape. A newly captured fox is known to have torn at the wooden door of his cage in a frenzy until he dropped dead from exhaustion.

Belyaev (1979) and Belyaev et al. (1981) concluded that selection for tameness was effective despite the many undesirable characteristics associated with it. For example, the tame foxes shed during the wrong season and developed black and white patterned fur. Changes were also found in their hormone profiles, and the monestrous (once a year) cycle of reproduction was disturbed. The tame foxes would breed at any time of the year. Furthermore, changes in behavior occurred simultaneously with changes in tail position and ear shape, and the appearance of a white muzzle, forehead blaze, and white shoulder hair. The white color pattern on the head is similar to many domestic animals (Belyaev, 1979) (Figs. 1.1 and 1.2). The most dog-like foxes had white spots and patterns on their heads, drooping ears, and curled tails and looked more like dogs than the foxes that avoided people. The behavioral and morphological (appearance) changes were also correlated with corresponding changes in the levels of sex hormones. The tame foxes had higher levels of the neurotransmitter serotonin (Popova et al., 1975). Serotonin is known to inhibit certain kinds of aggression (Belyaev, 1979). Serotonin levels are increased in the brains of people who take Prozac (fluoxetine).



FIGURE 1.1 Wild-type fox before Belyaev started selective breeding for tameness. *Reprinted from* Journal of Heredity *by permission of Oxford University Press.*



FIGURE 1.2 Selecting for tameness for many generations altered the body shape and coat color pattern. Foxes selected for tameness resembled dogs.

The fox experiments started by Dmitry Belyaev and Lyudmila Trut in the late 1950s are still ongoing. Researchers have bred both tame and aggressive foxes. Genomic sequencing indicates that they have distinct genetic profiles for genes involved in brain development (Rosenfield et al., 2019; Kukekova et al., 2018). Lord et al. (2020) claim that the fox experiments are not relevant for studying domestication because the original fur farm foxes had already been selected to breed in captivity and for coat color. They were not

truly wild foxes. One can argue about this, but the original Belyaev (1979) experiments clearly show that selection for the single behavioral trait of tameness resulted in physical changes in the animal that were not related to behavior.

Basic genetic mechanisms

Since the first and second editions, there has been a huge increase in research on genetic mechanisms. By the time this book is published, some of the material on genetic mechanisms may be obsolete. However, behavior studies reviewed in this book will remain useful to scientists working to discover new genetic mechanisms. The classical genetic concepts of recessive and dominant traits discovered by Gregor Mendel explain only a small fraction of the genetic factors affecting inheritance. It is beyond the scope of this book to provide an in-depth review of genetic mechanisms, instead, we describe key principles that will make it easier for the nongeneticist to read and understand the latest papers. Life is complicated. Increasingly complex non-Mendelian genetic mechanisms are being discovered (Schoenfelder and Fraser, 2019; Harich et al., 2020). They are best viewed as networks of information (Hayden, 2012). Below is an outline of some basic genetic mechanisms that produce changes in the appearance and behavior of animals.

Single-nucleotide polymorphisms

An single-nucleotide polymorphism (SNP) is a single code change in a single base pair of DNA. Guryev et al. (2004) state that SNPs are a major factor in genetic variation. In some Mendelian diseases, single SNPs or multiple SNPs are involved in disease inheritance (Kong et al., 2009; Shastry, 2002).

Repeats

Repeats are also called tandem repeats, single sequence repeats, or genetic stutters. Repeats are sequences of DNA code repeated more than once, and the number of repeats can vary within a gene. The number of repeats can determine many traits ranging from the length of a dog's nose to variations in brain development (Fondon and Garner, 2004; Fondon et al., 2008). Marshall et al. (2021) state that repeats are involved in both normal function and disease. Newer genomic sequencing methods will facilitate counting repeats.

Copy number variations

Copy number variations (CNVs) are rearrangements of genetic code. It is likely CNVs contribute greatly to genetic variation by modifying genetic

expression (Chaignat et al., 2011; Henrichsen et al., 2009). CNVs are often spontaneous (de novo) mutations not inherited from the parents. The many types of CNVs range from translocation of pieces of genetic code to deletions of genetic code or to extra copies of code. Twelve percent of the human genome is in copy number variable regions of the genome (Redon, 2006). CNVs are very numerous in the brain and the immune system (Harich et al., 2020).

Jumping genes

Also called transposable elements (transposons), jumping genes are short segments of genetic material that transport themselves throughout the genome in a "cut and paste," or a "copy and paste" manner. Mikkelson et al. (2007) state that transposons are a "creative force" in the evolution of mammalian gene regulation. Jumping genes are more numerous in the brain than in liver or heart cells (Vogel, 2011).

Coding DNA

The very small percentage of the genome that specifically codes for proteins is used in the development of the animal. Until recently, only coding DNA was sequenced. Coding DNA is only 2% of the human genome (Marshall et al., 2021).

Noncoding DNA, also called regulatory DNA

In the 1980s, this was called junk DNA because it does not code for proteins. Researchers have discovered that noncoding DNA has a regulatory function and approximately 80% of the noncoding DNA is transcribed by RNA and has biochemical functions (ENCODE Project Consortium, 2012). Noncoding DNA is the "computer operating system" that directs the coding DNA. Noncoding DNA may be the gene's "project managers" that orchestrate and direct the sequence of building proteins (Saey, 2011). Chakravarti and Kapoor (2012) state that to understand the genes that code for proteins, the regulatory noncoding DNA needs to be understood. Some portions of noncoding DNA are highly conserved, and similar sequences occur in many different animals. Other portions of noncoding genome may rapidly evolve (Maher, 2012). Coding regions of DNA that direct the development of basic patterning of the body (hoxgenes) are highly conserved across many species from arthropods to mammals (Linn et al., 2008). Early pattern formation of the notochord and neural tube is also highly conserved (Richardson, 2012). In both plants and animals, the embryos of many species look similar during the mid-embryonic stage of development. The mid-stage of development is "dominated by ancient genes" (Quint et al., 2012). A basic principle is that

similar traits in a species originate from a highly conserved genetic code. Traits that have recently changed originate from newer code. Research shows that changes in noncoding DNA are drivers of evolutionary change. The human neocortex has a higher percentage of young genes expressed during fetal development compared to mice (Zhang et al., 2011). Transposable bits of code can make changes in regulatory DNA (Mikkelson et al., 2007). The fact that similar sections of noncoding DNA occur in many species indicates its important function. Changes in noncoding DNA during environmental changes cause stickleback fish to adapt by changing traits, such as body shape, skeletal armor, or the ability to live in salt or freshwater (Hockstra, 2012). Sections of noncoding regulatory DNA evolve and change along with the coding DNA (Jones et al., 2012). Studies in other animals also show a role for noncoding DNA mutations in the development of domestic animals (Anderson, 2012).

Exome

The exome is the DNA sequencing of all the protein-coding regions of the genome. The noncoding DNA is left out of the exome.

RNA transcriptome

The RNA transcriptome is the DNA code that is read and transcribed by RNA. Sequencing the genome indicates that the animal's genome contains a particular piece of genetic code. The transcriptome indicates whether or not the code was transcribed by RNA and expressed as either a protein or involved in regulatory functions.

De novo mutations

De novo mutations are random mutations that are not inherited. Common de novo mutations are CNVs, SNPs, and other changes in DNA code.

Quantitative trait loci

Quantitative trait loci (QTLs) are regions of DNA containing many nucleotide base pairs associated with continuous traits such as height or temperament. QTLs are not associated with simple discreet Mendelian traits such as hair and eye color. QTLs are associated with phenotypic traits influenced by many genes (polygenic). They have limitations and QTLs may fail to detect genetic code that has the opposite effect on the trait being studied (York, 2018).

Haplotypes

Haplotypes are a group of genes linked together and inherited as a group.

Epigenetics

An animal's DNA may contain a certain sequence of genetic code, but it may be locked out by epigenetic mechanisms. Environmental influences can either lock out sections of code or unlock them. For example, epigenetic mechanisms either upregulate (make more anxious) or downregulate the nervous system of rodents depending on how much the pregnant mother was stressed, or how often a rodent is attacked by another rodent (Nestler, 2011, 2012).

Lamarckism

Jean-Baptiste Lamarck (1744−1829) was a French naturalist remembered for a theory of inheritance of acquired characteristics, more commonly referred to as soft inheritance, Lamarckism, or the theory of use/disuse. Lamarck believed that animals could acquire a certain trait during their lifespan and that the trait could be passed to the next generation. Part of Lamarck's argument is actually supported by the field of epigenetics and other parts of his theories were wrong. One part of this branch of science is based on the proteins (histones) binding to the DNA, winding it into a small enough shape to fit into the cell (Probst et al., 2009). Chemicals cause histones to bind either tighter or looser. Certain influences during life can influence the tightening or loosening of histones, these changes are then passed on (Sarma and Reinberg, 2005). If a piece of genetic code is loose, it will be easy to read, if it is tighter, it will not be read. This changes the expression of the genes and to a large extent explains the difference between twins at older ages, even when their genes are exactly the same (Fraga, 2005; Poulsen et al., 2007). Another epigenetic modification that serves to regulate gene expression without altering the underlying DNA sequence is DNA methylation. In simple terms, DNA methylation acts to "turn on" or "turn off" a gene. The methylation "lock" prevents a section of the DNA code gene from being read and expressed. For example, maternal obesity before and during pregnancy in mice affects the establishment of body weight regulatory mechanisms in her baby. This is caused by methylation locks that lock out a section of the genetic code. Overweight mothers give birth to offspring who become even heavier, resulting in amplification of obesity across generations (Champagne and Curley, 2009).

Some evidence suggests that the environment can make lasting changes to the expression of genes via epigenetic mechanisms—changes that may be passed on to future generations (Crews, 2010). Studies in rats show that

epigenetics influences maternal behavior and the effect can be passed on from one generation to the next (Cameron, 2008). The offspring of rat mothers who display high levels of nurturing behavior such as licking and grooming are less anxious and produce less stress hormones, compared to the offspring of less nurturing mothers. In turn, the female offspring of nurturing mothers become nurturing mothers themselves. The effects of maternal behavior are mediated in part through epigenetic mechanisms. In people, if the father has early life stress as a child, he may pass on brain changes to his child (Karlsson et al., 2020). This may be via an epigenetic mechanism.

Brain genetics more complex than other traits

For centuries, intensive selection in dogs has narrowed the gene pool for traits such as body shape and type of coat. Researchers have discovered that only a few genomic regions control many dog appearance traits (Boyko et al., 2010). This is not true for behavior. The genetics that controls brain development is much more complex. Selective breeding experiments in foxes and other experiments involving selection for appearance traits show that those traits are sometimes linked to behavioral traits. Why does selecting for a calm temperament produce a black and white fox? When the first edition of this book was written, these unusually linked traits were unexplained. The long-running ENCODE project (2012), which is mapping the noncoding regions of DNA, may help provide answers. Regions of noncoding DNA are not always located adjacent to the piece of code it regulates. There are long-range interactions. Sanyal et al. (2012, Giammartino et al., 2020) state that regulatory elements and coding DNA are in complex three-dimensional networks. Maybe when long strands of DNA are folded up, the temperament and coat color regions are folded up beside each other. Schoenfelder and Fraser (2019) state that regulatory information gets transcribed when it is in physical contact with target genes.

A brief historical review of animal behavior study

This historical review is not intended to be completely comprehensive. Our objective is to discuss some of the early discoveries important for our current understanding of animal behavior, with particular emphasis on genetic influences on behavior in domestic animals.

Early in the 17th century, Descartes came to the conclusion “that the bodies of animals and men act wholly like machines and move in accordance with purely mechanical laws” (in Huxley, 1874). After Descartes, others undertook the task of explaining behavior as reactions to purely physical, chemical, or mechanical events. For the next three centuries, scientific thought on behavior oscillated between a mechanistic view that animals are “automatons” moving through life without consciousness or self-awareness

and an opposing view that animals had thoughts and feelings similar to those of humans.

In "On the Origin of the Species" (1859), Darwin's ideas about evolution began to raise serious doubts about the mechanistic view of animal behavior. He noticed animals share many physical characteristics and were one of the first to discuss variation within a species, both in behavior and in physical appearance. Darwin believed that artificial selection and natural selection were intimately associated. Darwin (1868) cleverly outlined the theory of evolution without any knowledge of genetics. In "The Descent of Man" (1871) Darwin concluded that temperament traits in domestic animals are inherited. He also believed, as did many other scientists of his time, that animals had subjective sensations and could think. Darwin wrote: "The differences in mind between man and the higher animals, great as it is, is certainly one of degree and not of kind."

Other scientists realized the implications of Darwin's theory on animal behavior and conducted experiments investigating instinct. Herrick (1908) observed the behavior of wild birds to determine, first, how their instincts are modified by their ability to learn, and second, the degree of intelligence they attain. On the issue of thinking in animals, Schroeder (1914) concluded: "The solution, if it ever comes, can scarcely fail to illuminate, if not the animal mind, at least that of man." It is evident that by the end of the 19th century, scientists studying animal behavior in natural environments learned that the mechanical approach could not explain all behavior.

Behaviorism

During the middle of the 20th century, scientific thought again reverted to the mechanical approach and behaviorism reigned throughout America. The behaviorists ignored both genetic effects on behavior and the ability of animals to engage in flexible problem-solving. The founder of behaviorism, Watson (1930), stated, "differences in the environment can explain all differences in behavior." He did not believe that genetics had any effect on behavior. In "The Behavior of Organisms," the psychologist Skinner (1958) wrote that all behavior could be explained by the principles of stimulus−response and operant conditioning.

The first author visited with Dr Skinner at Harvard University in 1968. Skinner responded to a question about the need for brain research by saying, "We don't need to know about the brain because we have operant conditioning" (Grandin and Johnson, 2005). Operant conditioning uses food rewards and punishments to train animals and shape their behavior. In a simple Skinner box experiment, a rat can be trained to push a lever to obtain food when a green light turns on, or to push a lever very quickly to avoid a shock when a red light appears. The signal light is the "conditioned stimulus." Rats and other animals can be trained to perform a complex sequence of

behaviors by chaining together a series of simple operant responses. Skinner believed that even the most complex behaviors can be explained as a series of conditioned responses.

However, in a Skinner box a rat's behavior is very limited. It's a world with very little variation, and the rat has little opportunity to use its natural behaviors. It simply learns to push a lever to obtain food or prevent a shock. Skinnerian principles explain why a rat behaves a certain way in the sterile confines of a 30×30 cm Plexiglas box, but they don't reveal much about the behavior of a rat in the local dump. Outside of the laboratory, a rat's behavior is more complex.

Instincts versus learning

Skinner's influence on scientific thinking slowed a bit in 1961 following the publication of "The Misbehavior of Organisms" by Brelands and Brelands. Their paper described how Skinnerian behavioral principles collided with instincts. The Brelands were trained Skinnerian behaviorists who attempted to apply strict principles of operant conditioning to animals trained at fairs and carnivals. Ten years before this classic paper, Brelands and Brelands (1951) wrote, "we are wholly affirmative and optimistic that principles derived from the laboratory can be applied to the extensive control of animal behavior under non laboratory condition." However, by 1961, after training more than 6000 animals as diverse as reindeers, cockatoos, raccoons, porpoises, and whales for exhibition in zoos, natural history museums, department store displays, fairgrounds, trade convention exhibits, and television, the Brelands wrote a second article featured in the Breland and Breland (1961), which stated, "our background in behaviorism had not prepared us for the shock of some of our failures."

One of the failures occurred when the Brelands tried to teach chickens to stand quietly on a platform for 10−12 seconds before they received a food reward. The chickens would stand quietly on a platform in the beginning of training. However, once they learned to associate the platform with a food reward, half (50%) started scratching the platform, and another 25% developed other behaviors, such as pecking the platform. The Brelands salvaged this disaster by developing a wholly unplanned exhibit involving a chicken that turned on a jukebox and danced. They first trained chickens to pull a rubber loop that turned on some music. When the music started, the chickens would jump on the platform and start scratching and pecking until the food reward was delivered. This exhibit made use of the chicken's instinctive food-getting behavior. The first author remembers as a young adult seeing a similar exhibit at the Arizona State Fair of a piano-playing chicken in a little red barn. The hen would peck the keys of a toy piano when a quarter was put in the slot and would stop when the food came down the chute. This

exhibit also worked because it was similar to a Skinner box in the laboratory. It also utilized the natural pecking behavior of the chicken.

The Brelands experienced another classic failure when they tried to teach raccoons to put coins in a piggy bank. Because raccoons are adept at manipulating objects with their hands, this task was initially easy. As training progressed, however, the raccoons began to rub the coins before depositing them in the bank. This behavior was similar to the washing behavior raccoons do as instinctive food-getting behavior. The raccoons at first had difficulty letting go of the coin and would hold and rub it. However, when the Brelands introduced a second coin, the raccoons became almost impossible to train. Rubbing the coins together "in a most miserly fashion," the raccoons got worse and worse as time went on. The Brelands concluded that the innate behaviors were suppressed during the early stages of training and sometimes for long into the training, but as training progressed, instinctive food-getting behaviors gradually replaced the conditioned behavior. The animals were unable to override their instincts and thus a conflict between conditioned and instinctive behaviors occurred.

Ethology

While Skinner and his fellow Americans were refining the principles of operant conditioning on thousands of rats and mice, ethology was being developed in Europe. Ethology is the study of animal behavior in natural environments. The primary concern of ethologists is instinctive or innate behavior (Eibi-Eibesfeldt and Kramer, 1958). Essentially, ethologists believed that the secrets to behavior are found in the animal's genes, and the way genes were modified during evolution to deal with particular environments. The ethological trend originated with Whitman (1898), who regarded behavioral reactions to be so constant and characteristic for each species that, like morphological structures, they may be of taxonomic significance. A similar opinion was held by Heinroth (1918, 1938). He trained newly hatched fledglings in isolation from adults of their own species and discovered that instinctive movements, such as preening, shaking, and scratching, were performed by young birds without observing other birds.

Understanding the mechanisms and programming of innate behavioral patterns and the motivation underlying behavior is the primary focus of ethologists. Konrad Lorenz (1939, 1965, 1981) and Niko Tinbergen (1948, 1951) cataloged the behavior of many animals in natural environments. Together they developed the ethogram. An ethogram is a complete listing of the behaviors an animal performs in its natural environment. The ethogram includes both innate and learned behaviors.

An interesting contribution to ethology came from studies on egg-rolling behavior in the graylag goose (Lorenz, 1965, 1981). When a brooding goose notices an egg outside her nest, Lorenz observed that an instinctive program

triggers the goose to retrieve it. The goose fixates on the egg, rises to extend her neck and bill out over it, then gently rolls it back to the nest. This behavior is performed in a highly mechanical way. If the egg is removed as the goose begins to extend her neck, she still completes the pattern of rolling the nonexistent egg back to the nest. Lorenz (1939) and Tinbergen (1948) termed this a "fixed action pattern." Remarkably, Tinbergen also discovered that brooding geese can be stimulated to perform egg rolling on such items as beer cans and baseballs. The fixed action pattern of rolling the egg back to the nest can be triggered by anything outside the nest that even marginally resembles an egg. Tinbergen realized that geese possess a genetic-releasing mechanism for this fixed action pattern. Lorenz and Tinbergen called the object that triggers the release of a fixed action pattern "sign stimuli." When a mother bird sees the gaping mouth of her young, it triggers the maternal feeding behavior and the mother feeds her young. The gaping mouth is another example of sign stimuli acting as a switch that turns on the genetically determined program (Herrick, 1908; Tinbergen, 1951).

Ethologists also explained the innate escape response of newly hatched goslings. When goslings are tested with a cardboard silhouette in the shape of a hawk moving overhead, it triggers a characteristic escape response. The goslings will crouch or run. However, when the silhouette is reversed to look like a goose, there is no effect (Tinbergen, 1951). Several members of the research community doubted the existence of such a hard-wired instinct because other scientists failed to repeat these experiments (Hirsch et al., 1955). Canty and Gould (1995), repeated the classic experiments and explained why the other experiments failed. First, only goslings under 7 days old respond to the silhouette. Second, a large silhouette, which casts a shadow, must be used. Third, goslings respond to the perceived predator differently depending upon the circumstances. For example, birds tested alone try to run away from the hawk silhouette and birds reared and tested in groups tend to crouch (Canty and Gould, 1995). Nevertheless, fear is likely to be the basis of the response. Ducklings were shown to have higher heart rate variability when they saw the hawk silhouette (Mueller and Parker, 1980). Research by Balaban (1997) indicates that species-specific vocalizations and head movements in chickens and quail are controlled by distinct cell groups in the brain. To prove this, Balaban transplanted neural tube cells from developing quail embryos into chicken embryos. Chickens hatched from the transplanted eggs exhibited species-specific quail songs and bobbing head movements.

Do similar fixed action patterns occur in mammals? Fentress (1973) conducted an experiment on mice that clearly showed that animals have instinctive species-specific behavior patterns that do not require learning. Day-old baby mice were anesthetized and had a portion of their front legs amputated. Enough of the leg remained that the mice could easily walk. The operations were performed before the baby mice had fully coordinated movements so

there was no opportunity for learning. When the mice became adults, they still performed the species-specific face-washing behavior; normal mice close their eyes just before the foreleg passing over the face, and in the amputees, the eye still closed before the nonexistent paw hit it. The amputees performed the face-washing routine as if they still had their paws. Fentress (1973) concluded that the experiment proved the existence of instincts in mammals.

Two years after Breland's article, Jerry Hirsh (1963) at the University of Illinois, wrote a paper emphasizing the importance of studying individual differences. In it he wrote, "Individual differences are no accident. They are generated by properties of organisms as fundamental to behavior science as thermodynamic properties are to physical science."

Ethology and behaviorism provide tools to study emotions and behaviors

Both the behaviorists and the ethologists avoided the question of whether or not animals had emotions. They both developed a strictly functional approach to the motivations of behavior (DeWaal, 2011). Until relatively recently, most behaviorists and ethologists did not get involved with neuroscience. A review of the neuroscience literature makes it clear that emotional systems in the brain drive behavior. The research tools provided by the disciplines of both ethology and behaviorism are essential to further our understanding of animal behavior. Ethology provides the methodology for studying animals in complex environments. Bateson (2012) discusses the need to study freely moving animals. Animal behavior is more complex in natural settings or on a farm. Lawrence (2008) reviewed the behavior literature and determined that domestic animal research was changing from studying the basic biology of domestic animal behavior to studying animal behavior related to specific animal welfare issues. Lawrence (2008) warns that too narrow a focus on specific welfare concerns may be detrimental to answering broader welfare issues such as the subjective state of animals.

Neuroscience and behavior

Modern neuroscience supports Darwin's view on emotions in animals. All mammal brains are constructed with the same basic design. They all have a brainstem, limbic system, cerebellum, and cerebral cortex. The cerebral cortex is the part of the brain used for thinking and flexible problem-solving. The major difference between the brains of people and animals is in the size and complexity of the cortex. The emotional systems serving as drivers for behavior are located in the subcortex and are similar in all mammals (Panksepp, 2011; Montag and Panksepp, 2017). Primates have a larger and more complex cortex than a dog or a pig. Pigs have a more complex cortex

than a rat or a mouse. Furthermore, all animals possess innate species-specific motor patterns that interact with experience and learning in determining behavior. Certain behaviors in both wild and domestic animals are governed largely by innate (hard-wired) programs. Behaviors for copulation, killing prey, nursing young, and nest building tend to be more instinctual and hard-wired. Experience and learning play a larger role in behaviors that require more flexibility such as finding food, social interactions, and hunting.

Another basic principle to remember is that animals with large, complex brains are less governed by innate behavior patterns. For example, bird behavior is governed more by instinct than that of a dog, whereas an insect would have more hard-wired behavior patterns than a bird. This principle was clear to Yerkes (1905) who wrote:

Certain animals are markedly plastic or voluntary in their behavior, others are as markedly fixed or instinctive. In the primates, plasticity has reached its highest known stage of development; in the insects, fixity has triumphed, instinctive action is predominant. The ant has apparently sacrificed adaptability to the development of the ability to react quickly, accurately, and uniformly in a certain way. Roughly, animals might be separated into two classes: those that are in a high degree capable of immediate adaptation to their conditions, and those that are apparently automatic as they depend upon instinct tendencies to action instead of upon rapid adaptation. Since the publication of the second edition, researchers have embraced the concept of personality in animals (Cabrera et al., 2021; Finkelmeier et al., 2018). Emotions in animals are now being researched and discussed (Kremer et al., 2020; Burghardt, 2019). This is a major shift compared to the early 1990s. At this time, journal article reviewers forced the first author to remove the word *fear* from a cattle behavior paper. It had to be replaced with agitated (Grandin, 1993a,b).

Emotional systems motivate behavior

Great strides have been made in understanding how genetic factors affect behavior when scientists started by looking at brain systems that control emotions. This is the starting point for making it possible to sort out many conflicting results in behavioral studies. One big problem is that different words are used by different researchers to describe the same emotions (O'Malley et al., 2019).

The neuroscientist Jaak Panksepp outlined the major emotional systems located in the subcortical areas of the brain. The four main emotions are FEAR, RAGE, PANIC (separation distress), and SEEKING (novelty seeking) (Panksepp, 2005, 1998; Morris et al., 2011). He also listed three additional emotional systems of LUST, CARE (mother−young nurturing behavior), and PLAY. Each primary system is associated with a genetically

based subcortical brain network. Panksepp (2011) and Montag and Panksepp (2017) defined the basic emotional circuits of mammalian brains:

FEAR: An emotion induced by a perceived threat that causes animals to move quickly away from the location of the perceived threat, and sometimes hide. Fear should be distinguished from emotion anxiety, which typically occurs without any certain or immediate external threat. Some examples of fear are reactions to exposure to sudden novelty, startle responses, and hiding from predators. Fear is sometimes referred to as behavioral reactivity, behavioral agitation, or a highly reactive temperament. This emotion is often referred to as Bold (low fear) and shy (high fear) (O'Malley et al., 2019; Laine and Oers, 2017).

PANIC: Separation distress is an emotional condition in which an individual experiences excessive anxiety regarding separation from either home or from other animals that the individual has a strong emotional attachment to. One example of separation distress is a puppy or lamb vocalizing when it is separated from its mother. The PANIC system may also be activated when a single cow is separated from her herd. Sometimes referred to as social isolation stress or high social reinstatement behavior.

RAGE: A feeling of intense anger. Rage is associated with the fight-or-flight response and is activated in response to an external cue such as frustration or attempts to curtail an animal's activity. RAGE is the emotion that enables an animal to escape when it is in the jaws of a predator. In the research literature, it is also called aggression.

SEEKING: The seeking system (novelty seeking) in the brain motivates animals to become extremely energized to explore the world but is not restricted to the narrow behavioristic concept of approach, or the pleasure/reinforcement system. Seeking is a broad action system in the brain that helps coordinate feelings of anticipation, eagerness, purpose and persistence, wanting, and desire. This system promotes learning by urging animals to explore and to find resources needed for survival. A dog that excitedly sniffs and explores every room when turned loose in a strange house is an example of high SEEKING in an animal. In many behavior studies, this emotion is referred to as exploration (O'Malley et al., 2019). Open-field tests where an animal is placed along in an arena may be difficult to interpret from an emotional standpoint. Is the behavior in the open field motivated by PANIC (separation stress), FEAR, or SEEK? Perals et al. (2017) warn that other behavioral tests may be required to separate the traits of exploration and shyness (Fear).

Exciting new research has separated the variable of true novelty seeking, from activity or separation distress. Mice were exposed in an open field to both a familiar and a novel object (Farahbakhsh and Siciliano, 2021; Ahmadlou et al., 2021). This makes it possible to separate the attraction to novelty from the other variables. These researchers may have discovered a new circuit in the brain that is associated with novelty seeking.

LUST: The lust system in the brain controls sexual desire or appetite. Sexual urges are mediated by specific brain circuits and chemistries that overlap but are distinct between males and females and are aroused by male and female hormones.

CARE: The maternal nurturing system that assures that parents take care of their offspring. Hormonal changes at the end of pregnancy activate maternal urges that promote social bonding with the offspring.

PLAY: A key function of the play system is to help young animals acquire social knowledge and refine subtle social interactions needed to thrive. One motivation for PLAY is the dopamine-energized SEEKING system.

The existence of these emotional structures is well documented, and review articles on this research can be found in Morris et al. (2011), Burgdorf and Panksepp (2006), and Panksepp (2011). Direct electrical or chemical stimulation of specific subcortical structures elicits emotional responses. The brain circuits controlling fear and seeking in animals have been extensively mapped (LeDoux, 2000; Reynolds and Berridge, 2008).

Confusion of emotional systems may confound studies

In the behavior literature, many inconsistencies exist in papers on novelty seeking and fear. In many studies, novelty seeking may be confused with other emotional systems when terms such as activity level or emotional reactivity are used. Confusion may also exist between FEAR and PANIC when the term reactivity is used. The FEAR and PANIC (separation distress) systems have totally different functions. Fear keeps an animal away from danger and the PANIC system prevents the offspring from getting separated from its mother and helps to keep social groups together. Some scientists assume that an open-field test only evaluates fearfulness in animals (see Chapter 4). An animal alone in an open field may be reacting to separation from its mother or the social group. Confusing fear and separation distress is more likely when herding and flocking animals are tested in an open field, compared to animals that live a more solitary life. A term that is often used for describing behavior in an open field test is activity. It is likely that the motivation for activity is misinterpreted in many studies. Another big confusion in the scientific literature is behavior labeled personality. Zadar et al. (2017) state that tests for coping style did not correlate with personality assays. It is likely that the emotional systems are being mixed up. Panksepp's framework of the seven emotional systems may help sort out conflicting results in the scientific literature.

Research by Reynolds and Berridge (2008) has shown that the emotional traits of seeking novelty and fear are both controlled in a structure in the brain called the nucleus accumbens. When one end of the nucleus accumbens is stimulated, the animal becomes fearful. Stimulating the other end turns on

seeking (Faure et al., 2008). There is a mixture of fear and seeking receptors in the middle portion of the nucleus accumbens. The discovery of this function for the nucleus accumbens may explain the "curiously afraid" behavior we observed in cattle. Cattle curiously approach a novel clipboard laid on the ground, but when the wind flaps the paper, they fearfully jump back. When the paper stops moving, the cattle approach it again. The nucleus accumbens may be in SEEK mode when cattle voluntarily approach and switches into a fear mode when the paper suddenly moves.

Genetics and emotional systems

Research clearly shows that genetic factors have a very strong effect on both fearfulness and novelty seeking (Campler et al., 2008; Clinton et al., 2007; Stead et al., 2006). Maternal factors had little effect on novelty seeking in rats (Stead et al., 2006). Cross-fostering also had little effect, which shows that novelty seeking is highly heritable. Animals that are high seekers have more dopaminergic activity in the nucleus accumbens (Dellu et al., 1996). In addition to the heritable component of fearfulness, environmental influences also have an effect on fearfulness. Stressful treatment of either a pregnant mother or her offspring can upregulate the fear system. Lemos et al. (2012) propose that severe stress can disable the appetitive system in the nucleus accumbens. To state it more simply, stress can break the animal's SEEK function and it will no longer explore. Other emotional systems are also subject to both genetic and environmental influences. The PANIC (separation distress) system is highly heritable. In sheep, separation distress measured by isolating a single animal and measuring how many times it bleats (vocalizes) shows that vocalization is highly heritable (Boissy et al., 2005). Strength of the sex drive is also heritable. When the first Chinese pig breeds were imported into the United States, caretakers observed that boars were more highly motivated to mate compared to the United States and European commercial pigs. The Chinese pig was bred for large litter size and low levels of meat production.

The brain circuits that drive behavior are complicated. Bendesky et al. (2017) report that parental care behavior in mice has four separate brain circuits for licking their pups, retrieving their pups, huddling, and nest building. There is also increasing evidence that genetic factors that influence behavior in animals are also present in people. There is an interesting paper titled *Solitary Mammals as a Model for Autism* that reviewed genetic similarities between people with autism and animals that lead a more solitary life such as panthers or leopards (Resar, 2013). Two other studies showed that cattle temperament measured with a flight speed test was associated with genes for susceptibility of autism (Costilla et al., 2020; Chen et al., 2019). An extensive review article by Amanda et al. (2021) reported that half of the genes associated with farm animal behavior have also been identified as related to

behavioral and neuronal disorders in people. The same genes that make dogs friendly are also associated with Williams–Beuren syndrome in people (Von Holdt, et al., 2017). These studies clearly show that the same genetic codes are associated with emotions in both people and animals. Why would genes that are associated with variations in animal behavior be associated with brain disorders in humans? Maybe it is due to having a more complex brain. Sikela and Searles (2018) have a paper that may help explain this. It is titled *Genomic Tradeoffs: Are Autism and Schizophrenia the Steep Price for a Human Brain?* Building a huge human brain from stem cells that rapidly multiply may create more opportunities for mistakes during development. This may explain why genes associated with animal behavior would also be associated with brain disorders in humans.

Interactions between genetics and experience

Some behavior patterns are similar between different species, and some are found only in a particular species. For example, the neural programs that enable animals to walk are similar in most mammals (Melton, 1991; Grillner, 2011). On the other hand, courtship rituals in birds are very species-specific (Nottebohm, 1977). Some innate behavior patterns are very rigid and experience has little effect on them. Other instinctive behaviors can be modified by learning and experience. The flehmann, or lip curl response of a bull when he smells a cow in estrus, and the kneel-down (lordois) posture of a rat in estrus are examples of rigid behaviors. Suckling by newborn mammals is another example of a hard-wired behavioral system. Suckling behavior does not vary. Newborn mammals suckle almost everything put in their mouth.

An example of an innate behavior affected by learning is burrowing behavior in rats. Boice (1977) found that wild Norway rats and albino laboratory rats both dig elaborate burrows. Learning has some effect on the efficiency of burrowing, but the configuration of the burrows was the same for both the wild and domestic rats. Albino laboratory rats dug excellent burrows the first time they were exposed to an outdoor pen. Nest building in sows is another example of the interaction between instinct and learning. When a sow is having her first litter, she has an uncontrollable urge to build a nest. Nest building is hard-wired and hormonally driven. Widowski and Curtis (1989) showed that injections of prostaglandin F_2a induce nest building in sows. However, sows learn from experience how to build a better nest with each successful litter.

Other behaviors are almost entirely learned. Seagulls are known to drop shellfish on rocks to break them open, while others drop them on the road and let cars break them open (Grandin, 1995). Many animals ranging from apes to birds use tools to obtain food. New Calendonian crows use complicated tools to obtain food and can solve problems other birds cannot (Weir

and Kacelnik, 2006). Griffin (1994) and Dawkins (1993) provide many examples of complex learned behaviors and flexible problem-solving in animals.

Innate behaviors used for finding food, such as grazing, scavenging, or hunting, are more dependent on learning than behaviors used to consume food. Sexual behavior, nesting, eating, and prey-killing behaviors tend to be governed more by instinct (Gould, 1977). The greater dependence on learning to find food makes animals in the wild more flexible and able to adapt to a variety of environments. Behaviors used to kill or consume food can be the same in any environment. Mayr (1974) called these different behavioral systems "open" or "closed" to the effects of experience. A lion hunting her prey is an example of an open system. The hunting female lion recognizes her prey from a distance and carefully stalks her approach. Herrick (1910) wrote, "the details of the hunt vary every time she hunts. Therefore no combination of simple reflex arcs laid down in the nervous system will be adequate to meet the infinite variations of the requirements for obtaining food."

Interactions between instinctual hard-wired behavior and experience

Some of the interactions between genetics and experience have very complex effects on behavior. In birds, the chaffinch learns to sing its species-specific song even when reared in a sound-proof box where it is unable to hear other birds (Nottebohm, 1970, 1979). However, when chaffinches are allowed to hear other birds sing, they develop a more complex song. The basic pattern of the canary song emerges even in the absence of conspecific (flock-mate) auditory models (Metfessel, 1935; Poulsen, 1959). Young canaries imitate the song of adult canaries they can hear, and when reared in groups they develop song patterns that they all share (Nottebohm, 1977). Many birds, such as the white-crowned sparrow, chaffinch, and parrot, can develop local song dialects (Nottebohm et al., 1976; Adler, 1996). Sparrows are able to learn songs by listening to recordings of songs with either pure tones or harmonic overtones. Birds trained with harmonic overtones learned to sing songs with harmonic overtones, but 1 year later, 85% of their songs reverted back to innate pure tone patterns (Nowicki and Marler, 1988). Experiments by Mundinger (1995) attempted to determine the relative contribution of genetics and learning in bird songs. Inbred lines of roller and border canaries were used in this study along with a hybrid cross of the two. The rollers were cross-fostered to border hens and vice versa to control for the effects of maternal behavior. The roller and border males preferred to sing innate song patterns instead of copying their tutors. The hybrids preferred to learn some of both songs. Furthermore, canaries are capable of learning parts of an alien song but have a definite preference for their own songs. Comparing these animals to those in Brelands and Brelands (1961) exhibits, birds can be

trained to sing a different song, but genetically determined patterns have a strong tendency to override learning. In reviewing all this literature, it became clear that innate patterns in mammals can be overridden. Unfortunately, the animals tend to revert back to innate behavior patterns.

The paradox of novelty

Novelty is anything new or strange in an animal's environment. Novelty is a paradox because it is both fear-provoking and attractive. Paradoxically, it is most fear-provoking and attractive to animals with a nervous, excitable temperament. Skinner (1922) observed that pronghorn antelope, a very flighty animal, will approach a person lying on the ground waving a red flag. Kruuk (1972) further observed attraction and reaction to novelty in Thompson's gazelles in Africa. In small groups, Thompson's gazelles are most watchful for predators (Elgar, 1989). Animals that survive in the wild by flight are more attentive to novelty than more placid animals. Gazelles can also distinguish between a dangerous hunting predator and one that is not hunting. In Thompson's gazelles, the most dangerous predators attract the highest degrees of attraction. They often move close to a cheetah when the cheetah is not hunting. Furthermore, when predators walk through a herd of Thompson's gazelles, the size of the flight zone varies depending on the species of predator.

More recent research since the second edition may provide insights into why novelty is both scary and attractive. Christensen et al. (2020, 2021) found that horses that were more likely to explore a novel object could learn a discrimination task more quickly. In both birds and cattle, genetics has an effect on both the tendency to approach a novel object or avoid it (Hirata and Arimoto, 2018; Rozempolska-Rucinska et al., 2017). The explore (SEEK) trait and the FEAR treat are separate. Depending on the environment, the balance between SEEK or FEAR can be adjusted. Exploration facilitates learning but it may make an animal more vulnerable to predators. Fig. 1.3 shows the reaction of cattle to a novel box. Some animals approach it more quickly than others.

Reaction to novelty

Highly reactive animals are more likely to have a major fear reaction when confronted with sudden novelty. In domestic animals, examples of sudden novelty include being placed in a new cage, transport in a strange vehicle, an unexpected loud noise, or being placed in an open field. Using various experimental environments, Hennessy and Levine (1978) found that rats show varying degrees of stress and stress hormone levels proportional to the degree of novelty of the environment they are placed in. A glass jar is totally novel in appearance compared to a lab cage the animal is accustomed to.



FIGURE 1.3 The reaction of these cattle to a novel box placed in their pen clearly shows differences in their behavior. One animal immediately investigates the box, and the others are more reluctant to approach. Many of the other cattle are watching. It is likely that future research will find that the variation in their behavior can be explained by inherited differences in fearfulness and exploration (SEEK) traits.

Being placed in a glass jar was more stressful for rats than a familiar lab cage with no bedding.

Studies of reactions to novelty in farm animals have been conducted by Moberg and Wood (1982), Stephens and Toner (1975), and Dantzer and Mormede (1983). Calves show the highest degrees of stress when placed in an open-field test arena very dissimilar from their home pen (Dantzer and Mormede, 1983). Calves raised indoors were more stressed by an outdoor arena and calves raised outdoors were more stressed by an indoor arena. The second author is familiar with similar responses in horses. When horses are taken to the mountains for the first time, a well-trained riding horse accustomed to different show rings may panic when it sees a butterfly or hears a twig snapping on a mountain trail. Recent research with horses shows that when a familiar large complex object is rotated ninety degrees, a horse will react as if it is something new (Corgan et al., 2020). A child's brightly colored playset with a slide and a swing was used.

Genetic factors and the need for novelty

In mammals and birds, normal development of the brain and sense organs requires novelty and varied sensory input. Nobel prize-winning research of Hubel and Wiesel (1970) showed that the visual system is permanently damaged if kittens do not receive varied visual input during development. Dogs are more excitable when raised in barren and nonstimulating

environments (Melzack and Burns, 1965; Walsh and Cummins, 1975). Schultz (1965) stated; "when stimulus variation is restricted central regulation of threshold sensitivities will function to lower sensory thresholds." Krushinski (1960) studied the influence of isolated conditions of rearing on the development of passive defense reactions (fearful aggression) in dogs and found that the expression of a well-marked fear reaction depends on the genotype of the animal. In this experiment, Airedales and German shepherds were reared under conditions of freedom (in homes) and in isolation (in kennels). Krushinski (1960) found that the passive defense reaction developed more acutely and reached a greater degree in the German shepherds kept in isolation compared to the Airedales. In general, animals reared in isolation become more sensitive to sensory stimulation because the nervous system attempts to readjust for the previous lack of stimulation.

In an experiment with chickens, Murphy (1977) found that chicks from a flighty genetic line were more likely to become highly agitated when a novel ball was placed in their pen, but were more attracted to a novel food than birds from a calm line. Cooper and Zubek (1958) and Henderson (1968) found that rats bred to be dull greatly improved in maze learning when housed in a cage with many different objects. However, enriched environments had little effect on the rats bred for high intelligence. Greenough and Juraska (1979) found that rearing rats in an environment with many novel objects improves learning and results in increased growth of dendrites (nerve endings in the brain).

Pigs raised in barren concrete pens also actively seek stimulation (Grandin, 1989a,b; Wood-Gush and Beilharz, 1983; Wood-Gush and Vestergaard, 1991). Piglets allowed to choose between a familiar object and a novel object prefer the novel object (Wood-Gush and Vestergaard, 1991). Pigs raised on concrete are strongly attracted to objects to chew on and manipulate. The first author has observed that nervous, excitable hybrid pigs often chew and bite vigorously on boots or coveralls. This behavior is less common in the placid genetic lines of pigs. Although hybrid pigs are highly attracted to novelty, tossing a novel object into their pen will initially cause a strong flight response. Compared to calm genetic lines, nervous-hybrid pigs pile up and squeal more when startled. Pork producers report that nervous, fast-growing, lean hybrid pigs also tail-bite other pigs more often than calmer genetic lines of pigs (see Chapter 11). Tail biting occurs more often when pigs are housed on a concrete slatted floor that provides no opportunity for rooting.

Practical experience by both authors suggests that highly reactive horses are more likely to engage in vices such as cribbing or stall weaving when housed in stalls or runs where they receive little exercise. Denied variety and novelty in their environments, highly reactive animals adapt poorly compared to animals from calmer genetic lines (Huck and Price, 1975).

In summary, in both wild and domestic animals, novelty is both highly feared and necessary. Novelty is more desirable when animals can approach it voluntarily. Unfortunately, novelty is also fear-provoking when animals are suddenly confronted with it.

Temperament is not just about fear

Early research showed that animals as diverse as rats, chickens, cattle, pigs, and humans, genetic factors influence differences in temperament (fearfulness) (Royce et al., 1970; Blizard, 1971; Broadhurst, 1975; Fordyce et al., 1988; Fujita et al., 1994; Grandin, 1993b; Hemsworth et al., 1990; Kagan et al., 1988; Murphy, 1977; Murphey et al., 1980a; Reese et al., 1983; Tulloh, 1961). Trillmich and Hudson (2011) suggest that a broader area of animal personality needs to be studied. Today, the concept of temperament includes all the neurotransmitter systems that affect the strength of different emotional systems. Barr (2012) contains an outline of how behaviors such as fear and exploring are affected by different neurotransmitter systems. Some individuals are wary and fearful and others are calm and placid. Boissy (1995) stated, "fearfulness is a basic psychological characteristic of the individual than predisposes it to perceive and react in a similar manner to a wide range of potentially frightening events." In all animals, genetic factors influence reactions to situations that cause fear (Boissy and Bouissou, 1995; Davis, 1992; Kagan et al., 1988; Murphey et al., 1980b). Therefore temperament is partially determined by an individual animal's fear response. Rogan and LeDoux (1996) suggest that fear is the product of a neural system that evolved to detect danger and causes an animal to make a response to protect itself. Plomin and Daniels (1987) found a substantial genetic influence on shyness (fearfulness) in human children. Shy behavior in novel situations is considered a stable psychological characteristic of certain individuals. Shyness is also suggested to be among the most heritable dimensions of human temperament throughout the lifespan.

In an experiment designed to control for maternal effects on temperament and emotionality, Broadhurst (1960) conducted cross-fostering experiments on Maudsley reactive and nonreactive rats. These lines of rats are genetically selected for high or low levels of emotional reactivity. The results showed that maternal effects were not great enough to completely mask the temperament differences between the two lines (Broadhurst, 1960; Eysenck and Broadhurst, 1964). Maternal effects can affect temperament, but they are not great enough to completely change the temperament of a cross-fostered animal that has a temperament very different from that of the foster mother. In an extensive review of the literature, Broadhurst (1975) examined the role of heredity in the formation of behavior and found that differences in temperament between rats persist when the animals are all raised in the same environment.

In their study of genetic effects on dog behavior in a large sample, Fuller and Thompson (1978) found that "simply providing the same defined controlled environment for each genetic group is not enough. Conditions must not only be uniform for all groups, but also favorable to the development of the behavior of interest." In wartime Russia, Krushinski (1960) investigated the ability of dogs to be trained for the antitank service or as trail dogs trained to track human scent. The dogs were tied to a spike driven into the ground and the person who regularly looked after them let them lick from a bowl of food for a few moments then summoned the dog to follow the man as he moved 10−15 m away. The activity of each dog was measured with a pedometer for the next 2 minutes. The most active dogs were found to be the best antitank dogs. They were also fearless. In the antitank service, dogs were trained to run up to a tank and either run alongside of it or penetrate under the caterpillars of the tank. To do this, the dogs had to overcome their natural fear of a tank moving toward them at high speed. The less active dogs (as measured by the pedometer) were found to make the best trailer dogs. They slowly followed a trail and kept their noses carefully to the scent while negotiating the corners and turns on the trail. The more active dogs trailed at too high a speed and often jumped the corners and turn in the trail, which sometimes resulted in switching to another trail.

Mahut (1958) demonstrated an example of differences in fear responses between beagles and terriers. When frightened, beagles freeze and terriers run around frantically. In domestic livestock, measuring fear reactions during restraint or in an open-field test reveals differences in temperament both between breeds and between individuals within a breed (Dantzer and Mormede, 1983; Grandin, 1993a; Murphey et al., 1980b, 1981; Tulloh, 1961). Fearful, flighty animals become more agitated and struggle more violently when restrained for vaccinations and other procedures (Fordyce et al., 1988; Grandin, 1993a). Fear is likely to be the main cause of agitation during restraint in cattle, horses, pigs, and chickens. Recent studies on the genetic effects on behavior during transport handling and restraint of these animals are discussed in Chapter 4.

Species differences in emotional reactions to similar tests

In an open-field test, a single animal is placed in an arena (Hall, 1934). Rodents often stay close to the arena walls whereas cattle may run around wildly and attempt to escape. Possibly the reaction of the rodent is motivated by fear and the reaction of a single bovine may be PANIC (separation distress). The rodent has an instinctual fear of open spaces and stays close to the walls. The motivation is to avoid open spaces where it is likely to be seen by a predator. The bovine is motivated to rejoin its herdmates. The open-field test may be measuring different emotional systems in these two species. When fear is the motivator, a frightened animal may react in two

different ways. It may run around frantically and try to escape or in another situation, it may freeze and stay still. Chickens often freeze when handled by humans. Jones (1984) called this "tonic immobility." The chickens become so frightened they cannot move. In cattle, brahman *Bos indicus* cattle are more likely to go into tonic immobility than *Bos taurus* breeds such as Hereford. In wildlife, forceful capture can cause enough fear to sometimes inflict fatal heart damage. Wildlife biologists call this capture myopathy. In summary, much is known about the complex phenomenon of fear, but many questions still remain.

Biological basis of fear

Genetic factors influence the intensity of fear reactions. Genetic factors can also greatly reduce or increase fear reaction in domestic animals (Flint et al., 1995; Parsons, 1988; Price, 1984). Research in humans has clearly revealed some of the genetic mechanisms governing the inheritance of anxiety (Lesch et al., 1996). LeDoux (1992) and Rogan and LeDoux (1996) state that all vertebrates can be fear-conditioned. Davis (1992) recently reviewed studies on the biological basis of fear. Overwhelming evidence points to the amygdala as the fear center in the brain. A small bilateral structure located in the limbic system, the amygdala is where the triggers for "flight or fight" are located. Electrical stimulation of the amygdala is known to increase stress hormones in rats and cats (Matheson et al., 1971; Setckliev et al., 1961). Destroying the amygdala can make a wild rat tame and reduce its emotionality (Kemble et al., 1984). Destroying the amygdala also makes it impossible to provoke a fear response in animals (Davis, 1992). Blanchard and Blanchard (1972) showed that rats lose all of their fear of cats when the amygdala is lesioned. Furthermore, when a rat learns that a signal light means an impending electric shock, a normal response is to freeze. Destroying the amygdala eliminates this response (Blanchard and Blanchard, 1972; LeDoux et al., 1988, 1990). Finally, electrical stimulation of the amygdala makes humans fearful (Gloor et al., 1981). Animal studies also show that stimulation of the amygdala triggers a pattern of responses from the autonomic nervous system similar to that found in humans when they feel fear (Davis, 1992). Different types of fear are processed in separate neural circuits. Fear of predators, learned fear, and fear of conspecifics are some examples (Gross and Canteras, 2012). In birds, fear of a novel object is different from fear during tonic immobility. Fear can either elicit a response to freeze or flight to escape. Selection of quail for either high or low tonic immobility had little effect on reaction to a novel object (Saint-Dizier et al., 2008). Since the publication of the first edition of this book, scientists have learned that fear is not a single emotional trait. Further research shows that adding a third variable of human handling indicates that fear has more than one independent dimension (Richard et al., 2010).

Heart rate, blood pressure, and respiration also change in animals when the flight or fight response is activated (Manuck and Schaefer, 1978). All these autonomic functions have neural circuits to the amygdala. Fear can be measured in animals by recording changes in autonomic activity. In humans, Manuck and Schaefer (1978) found tremendous differences in cardiovascular reactivity in response to stress, reflecting a stable genetic characteristic of individuals.

Fearfulness and the mothering instinct conflict

Fearfulness and the mothering instinct can conflict. This principle was observed first hand by the second author during his experience raising Queensland Blue Heeler dogs. Some researchers may say that this observation is just anecdotal and has no value. This observation is being kept in the third edition because observations are essential for forming a hypothesis for future research studies. Annie's first litter was a completely novel experience because she had never observed another dog giving birth or nursing pups. She was clearly frightened when the first pup was born. It was obvious she did not know what the pup was. However, as soon as she smelled it, her maternal instinct took over and a constant uncontrollable licking began. Two years later, Annie's daughter Kay had her first litter. Kay was more fearful than her mother and her highly nervous temperament overrode her innate licking program. When each pup was born, Kay ran wildly around the room and would not go near them. The second author had to intervene and place the pups under Kay's nose. Otherwise, they may have died, Kay's nervous temperament and fearfulness were a stronger motivation than her motherly instinct.

Nervous system reactivity changed by the environment

Raising young animals in barren environments devoid of variety and sensory stimulation will have a detrimental effect on the development of the nervous system. Both the older research reviewed below and the most recent research clearly show the benefits of an enriched environment for young animals. Young mice that are socially isolated for 2 weeks after weaning have social impairments and defects in the circuits in the prefrontal cortex of the brain (Yamamuro et al., 2020). Juvenile mice that have been bred to exhibit autistic behavior will have less anxiety and increased social behavior when they are raised in an enriched environment (Queen et al., 2020). A deprived environment can cause animals to be more reactive and excitable as adults. This is a long-lasting, environment-induced change in how the nervous system reacts to various stimuli. Effects of deprivation during early development are also relatively permanent. Melzack and Burns (1965) found that puppies raised in barren kennels developed into hyperexcitable adults. In one

experiment, deprived dogs reacted with "diffuse excitement" and ran around a room more than control dogs raised in homes by people. Presenting novel objects to the deprived dogs also results in "diffuse excitement." Furthermore, the EEGs of the kennel-raised dogs remained abnormal even after they were removed from the kennel (Melzack and Burns, 1965). Research by Simons and Land (1987) shows that the somatosensory cortex in the brains of baby rats does not develop normally if sensory input is eliminated by trimming their whiskers. A lack of sensory input made the brain hypersensitive to stimulation. The effects persisted even after the whiskers had grown back.

Emotional reactivity develops in the nervous system during early gestation. Denenberg and Whimbey (1968) showed that handling a pregnant rat can cause her offspring to be more emotional and explore less in an open field compared to control animals. This experiment is significant because it shows that handling the pregnant mother had the opposite effect on the behavior of the infant pups. Handling and stressing pregnant mothers changed the gestational environment of the fetus resulting in nervous offspring. However, handling newborn rats by briefly picking them up and setting them in a container reduced emotional reactivity when the rats became adults (Denenberg and Whimbey, 1968). The handled rats developed a calmer temperament.

The adrenal glands are known to have an effect on behavior (Fuller and Thompson, 1978). The inner portions of the adrenals secrete the hormones, adrenaline, and noradrenaline, while the outer cortex secretes the sex hormones androgens and estrogens (reproductive hormones); and various corticosteroids (stress hormones). Yeakel and Rhoades (1941) found that Hall's (1938) emotional rats had larger adrenals and thyroids compared to the nonemotional rats. Richter (1952, 1954) found a decrease in the size of the adrenal glands in Norway rats accompanied by domestication. Several line and strain differences have been found since these early reports. Furthermore, Levine (1968) and Levine et al. (1967) showed that brief holding of baby rats reduces the response of the adrenal gland to stress. Denenberg et al. (1967) concluded that early handling may lead to major changes in the neuroendocrine system.

Taming does not change nervous system reactivity

Adult wild rats can be tamed and become accustomed to handling by people (Galef, 1970). This is strictly learned behavior. Taming full-grown wild animals to become accustomed to holding by people will not diminish their response to a sudden novel stimulus. This principle was demonstrated by Grandin et al. (1995) in training wild antelope at the Denver Zoo for low-stress blood testing. Nyala are African antelope with a hair-trigger flight response used to escape from predators. During handling in zoos for

veterinary treatments, nyala are often highly stressed and sometimes panic and injure themselves. Over a period of three months, Grandin et al. (1995) trained nyala to enter a box and stand quietly for blood tests while being fed treats. Each new step in the training had to be done slowly and carefully. Ten days were required to habituate the nyala to the sound of the doors on the box being closed.

All the training and petting by zookeepers did not change the nyala's response to a sudden, novel stimulus. When the nyala saw repairmen on the barn roof, they suddenly reacted with a powerful fear response and crashed into a fence. They had become accustomed to seeing people standing at the perimeter of the exhibit, but the sight of people on the roof was novel and very frightening. Sudden movements, such as raising a camera up for a picture, also caused the nyala to flee.

Domestic versus wild and fear responses

Wild herding species show much stronger fear responses to sudden novelty compared to domestic ruminants such as cattle and sheep. Domestic ruminants have attenuated flight responses due to years of selective breeding (Price, 1984). Wild ruminants learn to adapt in captivity and associate people with food, but are more likely to become agitated and injure themselves when frightened by novel stimuli (Grandin, 1993b, 1997). Fear is more likely when they are prevented from fleeing by a fence or other barrier. Principles for training and handling all herding animals are basically similar. Training procedures used on flighty antelope or placid domestic sheep are the same. The only difference is the amount of time required. Grandin (1989) demonstrated this by training placid Suffolk sheep to voluntarily enter a tilting restraining device in one afternoon. The nyala at the Denver zoo took 3 months to train. Each new procedure had to be introduced in smaller increments to prevent an explosive flight reaction.

Neoteny

Neoteny is the retention of the juvenile features in an adult animal. Genetic factors influence the degree of neoteny in individuals. Neoteny is manifested both behaviorally and physically. In the forward to "The Wild Canids" (Fox, 1975), Conrad Lorenz adds a few of his observations on neoteny and the problems of domestication:

The problems of domestication have been an obsession with me for many years. On the one hand, I am convinced that man owes the life-long persistence of his constitutive curiosity and explorative playfulness to partial neoteny that is indubitably a consequence of domestication. In a curiously analogous manner does the domestic dog owe its permanent attachment to its master to a behavioral neoteny that prevents it from ever wanting to be a

pack leader? On the other hand, domestication is apt to cause an equally alarming disintegration of valuable behavioral traits and an equally alarming exaggeration of less desirable ones.

Infantile characteristics in domestic animals are discussed by Price (1984), Lambooij and van Putten (1993), Coppinger et al. (1993), Coppinger et al. (1993), and Coppinger et al. (1987). The shortened muzzle in dogs and pigs is an example. Domestic animals have been selected for a juvenile head shape, shortened muzzles, and other features (Coppinger and Smith, 1983). Furthermore, retaining juvenile traits makes animals more tractable and easy to handle. The physical changes are also related to changes in behavior.

Genetic studies point to the wolf as the ancestor of domestic dogs (Isaac, 1970). During domestication, dogs retained many infant wolf behaviors. For example, wolf pups bark and yap frequently, but adult wolves rarely bark. Domestic dogs bark frequently (Fox, 1975; Scott and Fuller, 1965). Wolves have hard-wired instinctive behavior patterns that determine dominance or submission in social relationships. In domestic dogs, the ancestral social behavior patterns of the wolf are fragmented and incomplete. Frank and Frank (1982) observed that the rigid social behavior of the wolf has disintegrated into "an assortment of independent behavioral fragments." Malamutes raised with wolf pups fail to read the social behavior signals of the wolf pups. Further comparisons found that the physical development of motor skills is slower in the malamute. Goodwin et al. (1997) studied 10 different dog breeds, ranging from German shepherds and Siberian huskies to bulldogs, cocker spaniels, and terriers. They found that breeds that retained the greatest repertoire of wolf-like social behaviors were breeds that physically resembled wolves, such as German shepherds and huskies. Barnett et al. (1979) and Price (1984) both conclude that experience may also cause animals to retain juvenile traits. Gould (1977) found that the effects of neoteny are determined by changes in a few genes that determine the timing of different developmental stages. On average, wolves are smarter than dogs on a test involving spatial orientation and pulling rope. All the adult wolves passed the most complex version of this test, but only five out of 40 German shepherds could do it (Hiestand, 2011).

Overselection for specific traits

There are countless examples in the medical literature of serious problems caused by continuous selection for a single trait (Dykman et al., 1969; Steinberg et al., 1994). People experienced in animal husbandry know that overselection for single traits can ruin animals. Good dog breeders have known this for centuries. Some traits that appear unrelated are in fact linked. Wright (1922, 1978) demonstrated this clearly by continuous selection for hair color and hair patterns in inbred strains of guinea pigs. Selection for

hair color and patterns resulted in decreased reproduction in all the strains. Furthermore, differences in temperament, body conformation, and the size and shape of internal organs were found. Belyaev (1979) showed that continuous selection for tameness in foxes reduced maternal behavior and caused neurological problems. Graded changes occurred in many traits over several years of continuous selection for tame behavior. Physiological and behavioral problems increased with each successive generation. In fact, some of the tamest foxes developed abnormal maternal behavior and cannibalized their pups. Belyaev et al. (1981) called this "destabilizing selection," in contrast to "stabilizing selection," found in nature (Dobzhansky, 1970; Gould, 1977).

There are countless examples in the veterinary medical literature of abnormal bone structure and other physiological defects caused by overselecting for appearance traits in dog breeds (Ott, 1996). The abnormalities range from bulldogs with breathing problems to German shepherds with hip problems. Scott and Fuller (1965) reported the negative effects of continuous selection for a certain head shape in cocker spaniels:

In our experiments, we began with what were considered good breeding stocks, with a fair number of champions in their ancestry. When we bred these animals to their close relatives for even one or two generations, we uncovered serious defects in every breed ... Cocker spaniels are selected for a broad forehead with prominent eyes and a pronounced "stop" or angle, between the nose and forehead. When we examined the brains of some of these animals during autopsy, we found that they showed a mild degree of hydroencephaly, that is, in selecting for skull shape, the breeders accidentally selected for a brain defect in some individuals. Besides all this, in most of our strains, only about 50% of the females were capable of rearing normal, healthy litters, even under nearly ideal conditions of care.

Overselection in livestock

In pigs and cattle, indiscriminate selection for production traits such as rapid gain and leanness result in more excitable temperaments in pigs (Grandin, 1994). The first author has observed thousands of modern lean hybrid pigs. Lean hybrids have a greater startle response and are more excitable and difficult to drive through races compared to older genetic lines with more back fat. Separating single animals from the group is also more difficult. Research shows that cattle with an excitable temperament have lower weight gains and more meat quality problems (Café et al., 2011; Silveira et al., 2012; Voisinet et al., 1997a,b). This research illustrates that selection away from a very excitable temperament would be beneficial. However, ranchers report that mothering ability is reduced by overselecting for extremely calm temperaments. There are always tradeoffs.

Links between different traits

Casual observations by the first author indicate that the most excitable pigs and cattle have long slender bodies and fine bones. Some lean hybrid pigs have weak legs and normally brown-eyed pigs now have blue eyes. Blue eyes are often associated with neurological problems (Bergsma and Brown, 1971; Schaible, 1963). Furthermore, pigs and cattle with large bulging muscles often have calmer temperaments compared to lean animals with less muscle definition. However, animals with double muscling (hypertrophy) have more excitable temperaments (Holmes et al., 1972). Double muscling is abnormal and may have opposite effects on temperament compared to normal muscling.

Deafness in pointer dogs selected for nervousness is another example of apparently unrelated traits being linked (Klein et al., 1988). There appears to be a relationship between thermoregulation and aggressiveness. Sluyter et al. (1995) found that wild mice selected for aggressiveness used larger amounts of cotton to build their nests than mice selected for low aggression. This effect occurred in both laboratory and wild strains of mice.

The complexity of genetic interactions continues to frustrate researchers using high-tech "knockout" gene procedures. Genes are knocked out in a gene-targeting procedure and prevented from performing their normal functions. Knockout experiments show that blocking different genes can have unexpected effects on behavior. In one experiment, superaggressive mice were created when genes involved with learning were inactivated (Chen et al., 1994). The mutant mice had little or no fear and fought until they broke their backs. In another experiment, knockout mutants demonstrated normal behavior until they had pups, which they failed to care for (Brown et al., 1996). In another experiment, Konig et al. (1996) disabled the gene that produces encephalin (a brain opioid substance) and found unexpected results. Enkephalin is a substance normally involved in pain perception. Mice deficient in this substance were very nervous and anxious. They ran frantically around their cages in response to noise. Traits are linked in unexpected ways and completely isolating single gene effects may be impossible. Researchers suggest using caution and being careful not to jump to conclusions about claims by those who say they found an "aggression gene" or a "maternal gene" or an "anxiety gene." To use an engineering analogy, one cannot conclude the "picture center" in a television set was found after cutting one circuit inside the set used to create the pictures. Gerlai (1996) and Crawley (1996) also warn that knocking out the same gene in two different species may have different effects on behavior. This is due to the complex interactions between many different genes.

Thirty-five years ago, behavior geneticists found that inheritance of behavior is complex. Fuller and Thompson (1978) concluded, "It has been found repeatedly that no one genetic mechanism accounts exclusively for a particular kind of behavior."

Transgenic mice

Since the first edition of this book was published, hundreds of papers have been published on mouse models of conditions such as autism and various behavioral extremes (Roullet and Crawley, 2011; Silverman et al., 2010). Models of high-novelty-seeking mice, timid mice, fearful mice, and obsessive-compulsive mice are used to study these conditions in humans (Lipkind et al., 2004). There are different behavioral tests for the hundreds of types of special mice. Crawley (2007, 2008) devoted an entire book to behavioral testing of transgenic mice. Eisener-Dorman et al. (2009) stated that knockout procedures do not always work correctly to produce the desired effects and are concerned that transgenic mice are not always what they were originally bred to be.

Random factors and noninherited effects on variation

Behavioral geneticists find it impossible to completely control variation in some traits. Gartner (1990) found that breeding genetically similar inbred lines of rats failed to stop weight fluctuations. Even under highly standardized laboratory conditions, body weights continued to fluctuate between animals. Pig breeders also observe that commercially bred hybrid lines of pigs do not gain weight at the same rate. Even in genetically identical animals, random unknown factors affect variability. In utero factors may be one cause, other causes are unknown. Dr. Daryl Tatum and students at Colorado State University found both body conformation and meat quality variation in 50% English (*Bos taurus*) and 50% Brahman (*Bos indicus*) cattle. Some animals showed more Brahman characteristics, with larger humps and longer ears. However, the body conformation was not half English and half Brahman, and meat characteristics varied as well. Cattle that looked more Brahman had tougher meat. The animals had about 10% variation from the body shape and meat characteristics of Brahman half-bloods.

Two new studies clearly show two other noninherited causes of variation. Linneweber et al. (2020) studied wiring variations in the visual system of insects. These variations can have a profound effect on behavior. Even if the genetics is the same, the neurons will always grow slightly differently. In people, monozygotic identical twins may have differences. These differences may be due to mutations that occur right before the developing embryo splits in two (Jonsson et al., 2021).

Concluding thoughts

Many animal breeders are moving away from selecting for a narrow range of production traits. There are always tradeoffs. When decisions are made from the genetic selection. This analogy might be helpful when making

decisions. When selecting for different traits, imagine that an animal is country, with a financial budget that can be divided between the economy (production) infrastructure (reproduction and bone structure) and military (immune function). If a large percentage of the money is allocated to the economy (production), then the infrastructure sector and the military may get less. This may result in poorer reproduction bone structure or immune function. Poor reproduction in a high-producing dairy cow is a good example.

There is a complex interaction between genetic and environmental factors which determine how an animal will behave. The animal's temperament is influenced by both genetics and learning. Another principle to remember is that changes in one trait, such as temperament, can have unexpected effects on other unrelated traits. Overselection for a single trait may result in undesirable changes in both behavioral and physical traits.

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Chapter 2

Behavioral genetics and animal domestication

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ABSTRACT
We start by defining domestication as a genetic process, whereby animals change phenotypically and genetically as a response to living under human supervision. This has caused a convergent set of phenotypic modifications across different species, through a combination of relaxed natural selection, increased human selection, and correlated responses. The genetic mechanisms underlying these processes are discussed in addition to genetic drift and inbreeding. The driving types of mutations are dealt with, as is how genes are organized in the genome to give rise to a specific genetic architecture facilitating selection responses. The different methods used to discover genes and mutations are divided into either top-down approaches, where one starts with the phenotype and uses mapping methods to find the causative genes, or bottom-up, where the polymorphism is the starting point and the phenotype the desired response variable. A number of examples of single genes and mutations that have been coupled to behavior variation are given, among them genes showing pleiotropic effects on pigmentation, and genes involved in the regulation of the effects of neuropeptides. Finally, the relatively novel area of behavioral epigenetics is treated, in particular with its possible relation to domestication effects.

Definition of domestication

When Darwin first formulated his ideas on evolution, domesticated plants and animals played a central role. The first chapter of "On the origin of species" was devoted to a discussion about the rapid selection responses occurring during domestication, affecting widely different aspects of animal phenotypes. Darwin noted that size, color, and shape could all be modified in a few generations when the selection pressure was sufficiently intense, and he also rightly noted that the behavior of domesticated animals differs from wild relatives in a number of aspects. Hence, Darwin saw domestication as a rapid evolutionary process, something that has received massive

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support from research during the last century, and behavior was conceived as a phenotype among others.

The basic principles underlying any evolutionary process are few. In essence, an evolutionary development of a trait relies on three conditions: The trait varies between individuals within the population (the variation principle), the variation has a genetic component (the heritability principle) and individuals with different variants of the trait contribute unequally to the gene pool of the next generation (the principle of natural selection). The phenotypes of animals undergoing domestication develop according to the same principles. What makes domestication special is the fact that humans exert a large part of the selection pressure in deciding which individuals will be most likely to have their genes propagated to the next generation.

Attempts to define domestication are often based on the different selection pressure as compared to the natural situation. Hence, Price (2002) suggested that it should be defined as the process whereby captive animals adapt to man and the environment he provides. Price further argued that this is achieved through genetic changes occurring over generations and through environmental stimulation and experiences during an animal's lifetime. This has become the most influential definition and is widely cited by researchers in the field. However, in this chapter, we will not consider the experiential effects, as these are shared with any animal in captivity (e.g., habituation to humans and other aspects of the captive environment), and as they are not transmitted to the next generation, they should not be included in a formal definition of domestication. However, epigenetic effects may partly bridge the gap between genetics and experience; this will be considered more closely later.

Hence, we suggest that a more fruitful definition should focus on the effects, which transfer across generations. Domestication is the process whereby populations of animals change genetically and phenotypically in response to the selection pressure associated with a life under human supervision. Hence, domestication is an evolutionary process, and it acts through three different pathways, in addition to natural selection, which of course continues also in captivity. Firstly, there is a relaxation of specific natural selection pressures, such as predation and starvation; secondly, there is an intensified human selection for preferred traits, for example, growth, appearance, and reproduction; thirdly, there is a development of traits that are genetically or functionally correlated to the ones selected, for example, increased relative gut length in fast-growing broilers (Jackson and Diamond, 1995, 1996).

These three processes have also been discussed at length by Price (2002). Our definition leads to the view that domestication should be viewed as a genetic process. Alleles, which ultimately increase the reproductive output—the fitness—of an individual, are favored and will therefore be

overrepresented in coming generations. But due to the third process, certain alleles may also "hitch-hike" by being genetically linked to the selected locus.

In this chapter, we will therefore focus on behaviorally relevant genes and mutations that may have been selected during domestication, and discuss their functions and the mechanisms whereby they affect behavior. But we will also discuss genetic architecture, that is, how genes are physically organized in the genome, as this will be an important piece of information for understanding correlated selection and how the different aspects of the domestication phenotype are controlled genetically.

The domesticated phenotype

A striking aspect of domestication is the emergence in various, unrelated species, of very similar phenotypic changes. The complex of convergent traits is often referred to as "the domestic phenotype" (Price, 2002). This involves changes in appearance, reproduction, and behavior, often occurring as a coherent complex similar across species. Although in recent times it has been expanded to include numerous traits, as it was originally conceived the core set of domestication traits is generally restricted to tameness, alterations in body size and composition, alterations in brain size and composition, and changes in coloration (Price, 2002; Wright, 2015), with good evidence that this more reduced "core" set of traits is generally ubiquitous with all or certainly most domestic animals (Wright et al., 2020).

The first part of the domestication phenotype relates to the appearance of the domesticated animals in relation to their ancestors. For example, domesticates are generally smaller and have modified and often reduced pigmentation. The size reduction can be seen both as an overall smaller body mass and reduced length of limbs. This can however be reversed by selective breeding, and many domestic breeds are in fact considerably larger than their wild progenitors. For example, despite most dog breeds being considerably smaller than wolves, a few actually exceed the weight and wither height of wolves (Mosher et al., 2007), and many horse breeds are considerably larger than their presumed ancestor (Clutton-Brock, 1999). Nevertheless, in the absence of specific selection for the opposite, the general pattern is that domestication causes animals to decrease in size.

There is also a disproportionate size modification of certain body parts. For example, legs tend to be shorter in domesticated animals, referred to as chondrodystrophy, and the skull tends to show a more or less drastic shortening from nose to neck, so-called brachycephaly (Clutton-Brock, 1999). Further examples of typical aspects of the domesticated phenotype are floppy ears, curled tails, and modifications of fur structure. Also in birds, similar analogous changes occur, including alterations of size, skull shape, leg length, pigmentation, and plumage morphology.

Behavior and physiology have undergone a range of changes. Most prominent, domesticated animals generally exhibit a greatly reduced fear of humans (Price, 2002), which seems to generalize to other aspects of fear as well (Campler et al., 2009). Furthermore, domesticates tend to have an overall reduced activity level and perform less explorative behavior, and they also show less rigid social structures and often less agonistic behavior (Schütz and Jensen, 2001). For example, free-range dogs are known often to form dynamically changing packs of 30–50 individuals, while wolves live in strictly closed kinship groups of usually fewer than 10. Domesticated animals usually develop faster and reach sexual maturity earlier than their wild relatives. For example, dogs usually become sexually mature before 1 year old, whereas wolves take twice that time (Boitani and Ciucci, 1995). Red Junglefowl females start laying eggs at about 25 weeks old, whereas modern layers are reproductive already 7 or 8 weeks earlier (Schutz et al., 2002).

Thus although the variation is large, mainly due to a high variety of selection pressures for different, human-controlled purposes, a relatively uniform set of phenotypic changes reoccur in many species as a consequence of domestication. So this begs the question: why do these changes keep occurring? Are they part of an adaptive process or solely caused by human preferences? In any case, which are the genetic mechanisms involved?

When discussing the domestication phenotype (often referred to as the domestication syndrome), it is important to note that there is some debate as to how extensive it is, or indeed if it is a genuine phenomenon. Although the most common definition comes from Price (1984, 2002), the original idea appears to actually be derived from plant biology (and is attributed to Albert Thellung). Darwin, for example, did not define a discrete set of domestication traits and was more interested in assessing how correlated selection results in varieties differing than in defining universal features of domestication. Recently, there has been some debate as to the ubiquity of the domestication syndrome (Lord et al. paper), partly resulting from more and more traits being considered a part of this syndrome.

Examples of the expansion of the domestication syndrome include changes to ear form, tail form, horn form, craniofacial morphology, and the like, despite these traits not initially considered by Price (1984, 2002). The issue with such traits is that while such traits are certainly part of the domestication phenotype in some species, they are not unique to all. More fundamentally, Lord et al. (2020a,b) also raise the issue that as we do not have the exact ancient populations that the domesticated species were derived from (and which are the progenitors of modern domesticated species), it is impossible to say how these traits have changed. They also raise other considerations regarding the timing of when domestication traits arise and how they are associated with tameness. However, these issues are more related to the genetic basis of domestication, rather than the domestication phenotype per se (Wright et al., 2020).

Evolutionary mechanisms in domestication

Although natural selection does not end when a population is domesticated—diseases, parasites, competition, and predation still exert a strong effect on which individuals will reproduce—a strong selection is carried out by humans. Still, the traits, which can be selected will depend on the trait variation and the heritability of this, just like in the natural population. Hence, variation, heritability, and selection remain the same driving forces during domestication as they are in the wild.

It is, therefore, reasonable to claim that domestication is an evolutionary process whereby populations overall adapt to the prevailing selection pressures. In that sense, the domesticated phenotype may largely be caused by an adaptive response to human protection. For example, the body size of the wild ancestors has been optimally adjusted to the natural selection pressures in their wild habitats, such as food availability and predation risk. When predation pressure decreases and food availability goes up, it may be an adaptive response to reduce the body size. This will in turn enable faster development and increase lifetime reproductive success, something that may also have been appreciated and exacerbated by early farmers. It has been argued that many of the different parts of the domestic phenotype are typical of adaptive responses to r-selection, as opposed to k-selection (Price, 2002).

In the same way, several behavioral responses appear to be adaptive. For example, reduced rigidity in tolerance of variations of social structures can save energy and reduce the risk of injuries in situations where conditions are more likely to be crowded with many individuals in a limited area. It can also be adaptive to save energy by not engaging in excessive exploratory behavior when the net benefits from this are outweighed by the fact that food is provided by humans.

In a systematic comparison between domesticated White Leghorn laying hens and their ancestors, the Red Junglefowl, Schütz and Jensen (2001) studied groups of birds reared and kept under identical conditions. The domesticates were, in general, less active, displayed less social behavior, and were less investigative. The latter was shown for example by a decreased level of so-called contra freeloading; given a choice between hidden and freely available food, the domesticates ate less than the Red Junglefowl from the food source requiring some searching, digging, and scratching. The behavior of the White Leghorns was interpreted as an adaptive response to life under human protection, where food is abundant, predation is less of a risk and crowding is common.

It has been argued that the reduced natural selection pressure under domestication should lead to an increase in trait variation (Price, 2002). The logic of the argument is that, in absence of directed selection, the fitness of individuals carrying suboptimal traits should be relatively higher, and the population distribution should be flattened. There is little actual evidence to support this when considering specific domestic populations. However, when

directed selection is applied to a specific trait, between-breed variation may be dramatic. Dog breeds, for example, vary in adult size from about 700 g to 70 kg, and chickens come in dwarf versions of less than 500 g adult size, as well as giant breeds, reaching 7–9 kg. However, within breeds, the variation is less pronounced and mostly does not appear to be larger than in a population of wild ancestors. Hence, the directed, human-controlled selection under domestication may be as strong as the natural selection in the wild, causing trait distributions to change in domesticated populations without significantly changing the variation.

Over the last decade or so, researchers have started to investigate the specific adaptive properties of domesticated behavior. In particular, the cognitive and communicative abilities of dogs have received large attention. In general, dogs have developed traits, which are absent in wolves, and which make them more suited to interact and communicate with humans (Chen et al., 2009). For example, they are better at responding to human cues such as pointing and gazing, and they can learn and understand large numbers of spoken words and put these into categories. This probably represents exceptional adaptations in a species, which has been selected for many thousand years for its ability to cooperate and interact properly with humans, but it demonstrates that the specific selection pressures under domestication may lead to novel adaptations within a limited evolutionary time. The extent of this adaptation is highlighted by the fact that dogs, when human selection is removed (such as during feralization and in stray dogs), do not begin to exhibit wolf-like social behavior. Free-ranging dogs have a very different social and reproductive system than their wolf ancestors; they mate promiscuously and all individuals reproduce (unlike in wolves, where normally only one pair in a pack raises offspring) and they do not form highly cooperative, stable packs (Boitani and Ciucci, 1995).

A widely discussed problem is how many genes are actually involved in causing the domesticated phenotype. It has been suggested that the reoccurrence of similar phenotypic changes in many different domesticated species could be indicative of a limited number of "master genes," perhaps only one, controlling a range of traits (Stricklin, 2001). As selection acts on this gene, for example, by reducing stress and flightiness, several or all of the domesticated phenotypes would develop as correlated responses.

An important study sparking suggestions such as these is the one carried out by Belyaev and coworkers in Russia during the 1960s (Trut, 1999). They selected farm foxes (silver foxes, descendants of the red fox) for tameness in a simple test, measuring the propensity to flee or attack when approached by the hand of an observer. The response on the selected trait was rapid, and within a few generations, the selected foxes were tame and behaved in a dog-like manner toward humans.

Interestingly, several other domestication-related traits increased in frequency among the tame foxes. This included loss of pigmentation, faster

ontogenetic development, curly tails, floppy ears, and compressed jaws. Belyaev himself suggested that this may have been the result of released selection pressure on many genes, controlled by one or few "master genes" that were directly selected upon (Trut, 1999).

Similar results were found in an experiment where the Belyaev approach was used to select rats of high and low fearfulness toward handling. The tamer rats had a generally dampened stress response, as shown in both physiological and behavioral variables (Albert et al., 2008). However, even though several genetic loci seem to affect more than one of the phenotypic traits, Albert et al. (2009, 2011) did not find any evidence of a major, domestication gene. Rather, genetic architecture and the combined effects of several physically linked genes may explain the correlated selection responses.

In a very similar study, focusing on Red Junglefowl (ancestors of all domestic chickens), Agnvall and colleagues measured correlated behavior responses to selection for increased tameness against humans over three generations (Agnvall et al., 2012). They found a moderate but significant heritability for reduced fear of humans ($h^2 = 0.17$) and also significant genetic correlations between fear of humans and, for example, foraging and exploration, and hatch weight. A range of different traits typical of the domesticated phenotype also evolved over relatively few generations. For example, the less fearful birds grew bigger, showed increased feed efficiency, had a smaller brain relative to body size, and had a more dominant social behavior (Agnvall et al., 2014, 2015, 2017; Katajamaa and Jensen, 2021). This indicates that selection for tameness is likely to cause correlated behavioral effects also in chickens and again suggests that genetic architecture of few and pleiotropic genes may control a large part of the domesticated phenotype.

What is interesting in the study of the genetic architecture of domestication is that when these differentially selected, or indeed wild and domestic, populations are then intercrossed, these trait correlations begin to erode (Albert et al., 2009; Wright et al., 2010; Johnsson et al., 2014; Wright, 2015). The implications of this are discussed in the section on genetic architecture, later.

Genetic mechanisms in domestication

All the standard genetic mechanisms that act on natural populations will also act on domestic populations, though some to a much greater and others to a much lesser degree. Genetic mechanisms affecting gene frequency can be largely broken down into systematic processes and dispersive processes (Falconer and Mackay, 1996).

Systematic processes affect gene frequency in both a predictable manner and direction and include migration, mutation, and selection. Dispersive processes have greater effects in smaller populations and are often unpredictable in their effects on populations. This is principally drifting, but also inbreeding and assortative mating can fall under this category. In terms of the latter two

(inbreeding and assortative mating) these affect not only gene frequencies, but more importantly genotype frequencies, as discussed below. Of these processes, selection, inbreeding, and drift are perhaps the factors with the greatest effect on domestic populations (Price and King, 1968). Migration (i.e., new gene flow into the population) is generally extremely tightly controlled in domestic animals, while mutation may provide some novel discrete Mendelian (single gene) phenotypes that are then selected upon. Quantitative traits will once again be relatively unaffected in the relatively short time frame (evolutionarily) of domestication (especially when one considers that some of the largest changes in phenotype have occurred in the most recent selection history of domestication). Assortative mating (whereby phenotypically similar animals mate/are mated with one another) will have an effect in domestic animals, though this will generally be due to selection for a specific phenotype, and will also often be closely related to inbreeding.

Selection

Selection can be further subdivided into artificial (both intentional and inadvertent), natural, and relaxed in the case of domestic animals.

Artificial selection: This can probably be thought of as both the strongest and most defining mechanism of domestication (Price and King, 1968). The effects of this can be extremely rapid, and even within even just a few generations of selection, large differences can occur. For example, both silver foxes (Belyaev et al., 1985, 1988) and rats (Albert et al., 2008, 2009) selected for tameness show a variety of behavioral and physiological responses after just a few generations, as discussed earlier. Chickens selected for differential growth show a similar marked response to selection (Carlborg et al., 2006). These are examples of intentional artificial selection, though unintentional artificial selection can also occur. Examples of this would be the coonstripe shrimp, *Pandalus danae*, inadvertently selected over 10 generations for a decrease in escape tail-flip response (this response lead to injury during handling (Marliave et al., 1993)) and for trout inadvertently selected for stress reduction to increase fecundity (Fevolden et al., 1991). One important difference to consider between artificial and natural selection has been put forward by Price (1999), in that artificial selection is "goal-oriented," whereby individuals with a desirable phenotype are then bred from (i.e., selection occurs before reproduction), whereas with natural selection an individual is only "assessed" through its representation in future generations (i.e., the number of offspring it produces).

Relaxed selection: As mentioned earlier, certain behavioral adaptations, principally foraging ability and predator avoidance, are no longer relevant to the survival of an animal in captivity. In the case of antipredation behaviors, in particular, there are associated costs with exhibiting these traits and relaxed pressure may therefore drive the traits in the opposite direction

(especially considering that wild populations with lower predation pressure exhibit these behaviors less). Kronenberger and Medioni (1985) showed that domestic mice preferred novel saccharin-water more than their wild counterparts. Such neophobia is often seen in wild-derived populations, but less so in domestic populations (Schütz and Jensen, 2001; Wright et al., 2006c).

This relaxation of selection could be expected to lead to an increase in phenotypic and genetic variability; however, this is often confounded with the limited genetic variability found in certain domestic populations. For example, laboratory strains often have limited genetic polymorphism. Animals tend to come from a limited founder population (e.g., mice—Bonhomme and Guenet, 1984) and therefore have greater genetic homogeneity. Using a new series of crosses between animals recently caught from the wild, Koide et al. (2000) derived a series of mouse strains that exhibited much greater polymorphism than standard laboratory strains, with several strains also diverging in assorted behavioral assays.

Natural selection: Although domestic animals are obviously subject to strong artificial selection, this does not mean that natural selection does not occur. Indeed, any selection that occurs in the captive environment that is not artificial is by definition natural (Hafez, 1962). Even those animals that are artificially selected for breeding may nevertheless fail to reproduce. This can be due to a myriad of factors, including social stress, parasitism, and in general a failure to cope with the captive environment. Those animals that cope best with this new environment will increase their reproductive potential. For example, wild-caught *Drosophila* populations that were maintained in captivity were found to exhibit rapid adaptation to the captive environment over nine generations (Frankham et al., 1999).

Not only that, but the artificial selection process itself, acting on certain traits may also lead to a reduction in reproductive potential. For example, dog breeds have long been known to show negative fitness effects due to selected characteristics [e.g., the ridge in ridgebacks is associated with sinus formation (Hillbertz et al., 2007), hip dysplasia in alsatians (Hedhammar et al., 1979)], growth is linked with decreases in reproduction in broilers (Gross and Siegel, 1985), and the rex hair color in rabbits leads to metabolic problems, disease susceptibility, and increased mortality (Müntzing, 1959). Generally, the extent of the occurrence of natural selection can be thought of as determined by the extent to which the environment allows the expression of species-typical traits (Spurway, 1955). Animals with very few preadaptations to this environment will therefore naturally be under greater natural selection as they are subjected to a strong, novel environment.

Inbreeding

Inbreeding, the process whereby related individuals assortatively breed together, leads to an increase in alleles identical by descent (Falconer and

Mackay, 1996). This can be typically measured by assessing the excess numbers of homozygotes in the population, due to substructure. In domestic populations, inbreeding is relatively common, especially in more modern selection practices, as fewer numbers of animals tend to get a far larger percentage of the matings. This is especially true when selection indices (Best Linear Unbiased Predictor analysis and the like) are used to calculate the most desirable individuals to breed from and therefore tend to increase the rate of inbreeding (Belonsky and Kennedy, 1988; Bijma and Woolliams, 2000) reviewed in Kristensen and Sørensen (2005). This can be a greater problem in certain domestic breeds than others.

For instance, the level of inbreeding in dogs has been shown to be extremely high (Calboli et al., 2008), whereas chicken breeds remain remarkably diverse, even in domestic breeds (Rubin et al., 2010). The general effects of inbreeding are commonly agreed to lead to inbreeding depression. In many appearances, the opposite of heterosis (or outbreeding vigor) is manifest in a decrease in general fitness (Lynch and Walsh, 1998). Although many such examples exist for a variety of animal models with rather variable results, as concerning domestication, sheep (Wiener et al., 1992, 1994) and dairy cows (Miglior et al., 1995; Smith et al., 1998) have been shown to exhibit effects in primary fitness. Inbreeding can also have negative effects on behavior. Courtship behavior in guppies (Mariette et al., 2006) and *Drosophila* (Sharp, 1984) have both been shown to be negatively affected by inbreeding. Parental behavior has also been found to be affected in mice (MARGULIS, 1998). The detrimental effects of inbreeding can also be seen in the numerous mechanisms to avoid inbreeding (e.g., nonselfing mechanisms in plants (Charlesworth and Charlesworth, 1979)) and behavioral mechanisms in animals (Pusey and Wolf, 1996).

The mechanism through which the negative effects of inbreeding act have traditionally been thought of as due to the effects of dominance between loci (Lynch and Walsh, 1998). If the effects were purely additive, they would be independent of the genetic background and there should be no effect of increased homozygosity. There are three general hypotheses for describing inbreeding depression—the (erroneously named) dominance hypothesis, the overdominance hypothesis, and the breakdown of epistasis hypothesis. The dominance hypothesis is in fact nothing to do with dominance but is related to the expression of deleterious recessives that are more likely to arise due to identity by descent (Davenport, 1908). The overdominance hypothesis relies on the fact that heterosis causes an increase in fitness, therefore a reduction in heterozygosity (as occurs during inbreeding) will lead to a reduction in fitness (Davenport, 1908). This hypothesis means variation is maintained in the absence of mutation, whereas with the dominance hypothesis a flux of deleterious mutations arise and then become fixed in the inbred individuals. The breakdown of epistasis is a more recent theory and relates to the web of epistatic interactions that potentially exist between

alleles (Templeton and Read, 1994). As these are disrupted in inbred individuals (especially if heterozygosity is required for these epistatic interactions), this could explain both heterosis (hybrid vigor) and inbreeding depression.

Drift

Drift is the name given to random fluctuations in gene frequency due to stochastic processes (Falconer and Mackay, 1996) and is generally most important in small populations (with domestic populations frequently having low effective population sizes). It is also most pronounced when substructure, for example, inbreeding, has occurred to further decrease the effective population size. In this instance, it can readily lead to a loss of allelic diversity and is therefore similar in effect to inbreeding. Founder effects (when limited numbers of individuals are used to generate the initial population) and bottle-necks (whereby only a limited number of individuals are used to generate the next generation at certain points) will also decrease this initial diversity (in the case of founder effects) or reduce it during their occurrence (in the case of bottle-necks) (Sunden and Davis, 1991).

Allelic diversity is important to long-term adaptation (Allendorf, 1986) and is lost more readily than heterozygosity. However, the same measures to avoid the loss of heterozygosity should also help with drift, for instance with the use of large founder populations with efforts made to maintain diversity (Ferguson et al., 1991) or back-crossing back to wild individuals (Vila et al., 2005). Effects of this loss of diversity are potentially somewhat equivocal, though generally thought to be negative. A study with bottle-necked *Drosophila* populations found a decreased tolerance to NaCl compared to control populations (Frankham et al., 1999), while effects on within-line variance (Avery and Hill, 1977) and variance in response (Wray and Hill, 1989) have been shown. However, Carson (1990) found no effects on variance after a bottle-neck event in *Drosophila* and house-fly populations.

The role of hybridization in domestication

Having considered the evolutionary forces that can act to create the domesticated phenotype, it is also of interest to note that hybridizations may also play a role in the creation and maintenance of a domesticated species. Hybridization can be a method by which additional variation is introduced into a species undergoing, or indeed, that is already, domesticated. Such hybridization may occur with the interbreeding with closely related species, or with wild individuals of the same species (Barton, 2013). With hybridization, favorable alleles can become integrated into the genome of a domesticated population, and then be the target of continued selection.

It is possible that people may have taken precautions against hybridization between domesticated animals and their wild ancestors. After all, when

selection had yielded animals with favorable traits, hybridization would risk destroying the breeding achievements. Tractable and friendly dogs run the risk of generating shy and more aggressive offspring if paired with wolves (Hansen Wheat et al., 2018), and chickens laying large and plentiful of eggs risk losing their production capacity if hybridized with ancestral Red Junglefowl (Wright et al., 2006b). Nevertheless, such hybridization has apparently been ubiquitous during domestication in several species and, in some cases, may have contributed favorable introgressions. In fact, some authors have posited that multiple repeated hybridizations with domestics and related wild species or populations have formed the backbone of many modern domestic species, with evidence for this being seen in the genetic architecture of domesticated traits (see later). For example, Grant et al. (Grant, 1981; Wright, 2015) posit the genetic architecture of domestication (comprising of modules of linked genes) is due to repeated introgressions to insert desirable traits, with these modules then enabling the rapid recapitulation of the domesticated phenotype.

In dogs, repeated hybridization appears to have occurred in several different populations, as revealed by extensive genome analyses (vonHoldt et al., 2010), and the same appears to be the case with pigs (Iacolina et al., 2018). However, it is not generally clear to what extent such hybridization events have contributed any favorable traits to the domesticated population, with an interesting exception: the yellow leg phenotype in domestic chickens.

Most chicken breeds across the world have yellow legs and beaks, whereas those of the ancestral Red Junglefowl are gray. A genome-wide selective sweep analysis revealed a strong signature of selection of the gene responsible for this difference, *BCO2* (Rubin et al., 2010). Further analyses showed that the domesticated variant is in fact a result of an early species hybridization event, as its origin can be traced with a high certainty to the closely related Gray Junglefowl (*Gallus sonneratii*) (Eriksson et al., 2008). The domesticated variant allows chickens to store carotenoids in the skin and then deposit those in the egg yolk, increasing the nutritional value of the eggs both for embryos and human consumers (Fallahshahroudi et al., 2019). This would have increased the fitness and utility of chickens that live with humans in conditions and regions where natural carotenoids are scarce in the available food.

Genetic architecture of domestication

The term genetic architecture refers to the location, number, and effect size of the number of loci (Quantitative Trait Loci, or QTL) that underpin the variation in domestication phenotypes (Falconer and Mackay, 1996). The strong directional selection associated with domestication can potentially lead to a distinctive genetic architecture (Andersson and Georges, 2004), while the form of this genetic architecture appears to be remarkably

consistent in both plant and animal species (Dingemanse et al., 2010). This architecture has been principally analyzed through QTL studies, whereby wild populations are crossed with domestic breeds, with the resulting hybrids (termed F1's) then either intercrossed together again or back-crossed to one of the parental populations. These individuals are then analyzed genetically, allowing the major effect loci to be identified positionally on the genome, and the relative strength of the effect (i.e., how much they influence the phenotype) estimated.

What all these diverse domesticated species appear to have in common is that the domestication traits themselves all seem to be grouped together in clusters or modules in the genome, rather than being randomly distributed (i.e., a QTL for increased growth may lie near a QTL for decreased anxiety behavior, rather than being very dispersed). Starting with domesticated plant species, beans (Koinange et al., 1996; Pérez-Vega et al., 2010), rice (Cai and Morishima, 2002), maize (Doebley and Stec, 1991, 1993), and sunflowers (Burke et al., 2002) all show this pattern. In animals, such modules are also once again seen when using similar wild × domestic crosses. For example, in the chicken, various behavioral, morphological, and life-history traits have all been shown to be grouped together in clusters (Schutz et al., 2002; Wright et al., 2006b, 2008, 2010, 2012). Similarly, a study using rat populations selected for high and low aggression also found similar modules composed of QTL affecting physiological and behavioral traits (Albert et al., 2009). Silver foxes differentially selected for tame and aggressive behavior have also been shown to exhibit correlations with other dog-like characteristics (including skull shape and color) (Trut, 1999; Hare et al., 2005). In a QTL analysis of these animals, which was restricted to behavior, overlaps between different behavioral measures were found (Kukekova et al., 2011). A cross between wild and laboratory zebrafish populations found correlations between growth and boldness behavior in the intercross generation (Wright et al., 2006a,c).

When such modules do occur a pertinent question is of course whether each module represents one general "domestication-locus" (i.e., all the domestication effects arise from a single pleiotropic locus), or whether they in fact comprised many different, but closely physically linked, loci. In the case of plants, there have been some examples where the actual causative genes and mutations have been identified, allowing this question to be addressed more accurately. Where individual genes have been identified in plants, these do indeed appear to have pleiotropic effects. The *Q* gene in wheat (Faris et al., 2003; Simons et al., 2006) and the gene *tb1* in maize (Doebley and Stec, 1993; Clark et al., 2004) being classic examples. However, with each of these examples, additional mutations and causative genes have also been identified in separate, but physically linked, genes (Doebley and Stec, 1991, 1993; Ji et al., 2006). In the animal examples, it is therefore tempting to suggest that these modules, similar to the pleiotropic

plant genes, represent genuine pleiotropy. However, this may not be the case—one of the main issues with QTL analysis is the large confidence intervals (CIs) when an F2 or backcross format are used, due to the relatively low number of recombinations that may have occurred between closely linked genes (Lander and Botstein, 1989; Lynch and Walsh, 1998). In this case, such large CIs will be unable to distinguish close linkage from pleiotropy without very large sample sizes.

As an illustration, in the chicken study by Wright et al. (Wright et al., 2010, 2012; Fogelholm et al., 2019, 2020; Henriksen et al., 2020; Höglund et al., 2020b), which analyzed a wide variety of behavioral (anxiety and fear-avoidance behaviors) (Johnsson et al., 2016a, 2018a; Fogelholm et al., 2019; Henriksen et al., 2020), morphological (growth and bone density) (Johnsson et al., 2015a, 2018b), and life-history (onset of sexual maturity and fecundity) traits (Wright et al., 2012; Johnsson et al., 2015b), a very large sample size and multivariate statistical analysis was used to attempt to dissect pleiotropy from the linkage. Whereas the central loci in the modules were shown to be indistinguishable from pleiotropy, those on the periphery were linked, rather than pleiotropic. A follow-up study using an advanced intercross (to increase the number of recombinations present in the cross) examined one module, which affected comb mass and bone allocation, and this was genuinely pleiotropic (Wright et al., 2010; Johnsson et al., 2012). Pooling these different results from domesticated species, it appears that these modules may contain central "cores" of pleiotropic loci, surrounded by more loosely linked loci.

This idea of loose-linkage was first alluded to by Grant (1981). He noticed that even with a strong domestication phenotype, multiple correlations between different aspects of the domestication phenotype would rapidly breakdown when reverse selected or cross-bred. Although he noticed this with several plant-based examples, the same is true with the correlations between different domestication characteristics breaking down rapidly during intercrossing (Albert et al., 2008, 2009; Wright et al., 2010). The uses of this in domestication would be to enable new phenotypes to be rapidly bred into a certain background (far harder if traits are generally all pleiotropic), and the preexisting domestic phenotype regained. Although this may be an advantage, as it allows desirable characteristics to be rapidly bred into the population and then the previous domesticated phenotype to be rapidly regained, it is unclear whether this ability would be specific to domesticated species (potentially explaining the rather limited numbers of domesticated species) or is a general feature of strong directional selection on phenotypic evolution, which is then exploited in the domestication process.

In addition to modularity being observed in the organization of loci affecting different aspects of the domestic phenotype, modularity can also be seen in genome organization and gene expression in general. Using the whole-genome expression variation, it appears that the differences between

wild and domestic populations are due to hundreds or thousands of gene expression changes (Rubin et al., 2007; Lai et al., 2008; Natt et al., 2012). Once again, these differentially expressed genes tend to be grouped together into modules that are once again close to one another. In effect what this means is that genes that lie physically close to one another tend to show the same expression pattern.

Examples come from microarray data (whole-genome expression profiles) using inbred lines, or recombinant inbred lines. What is striking is that in many of the microarray studies comparing wild and domestic populations (that show large-scale differential expression), the QTL architecture appears to be relatively simplistic. Litvin found the total number of transcriptional differences could be reduced down to a relatively small number of modules (Litvin et al., 2009), while Chesler et al. found a small number of QTL modulated large transcriptional gene expression sets that also correlated with behavior (Chesler et al., 2005). These gene modules may therefore represent pleiotropy or close linkage, with a single gene potentially leading to numerous changes in gene expression. In effect, the large-scale changes would be downstream effects of the initial causative change. Wild inbred lines also show this pattern, with differentially expressed genes grouped together in clusters in the genome (Ayroles et al., 2009). In these examples, it is once again hard to know if these represent multiple linked loci, genuine pleiotropy, or a combination of the two. Pleiotropic hotspots have also been discovered in other eQTL studies—see a review in Breitling et al. (2008), though there is some debate as to whether these are genuine or not, and how common they are (Breitling et al., 2008).

When considering the extent of pleiotropy in domestication, one of the most cited examples of this is the study by Belyaev. In this experiment, Belyaev and colleagues took 130 foxes and within ten generations of selection solely for tameness derived a population with many of the domestication traits seen in dogs (curly tails, floppy ears, altered craniofacial morphology), with this being seen as evidence for pleiotropy (Belyaev et al., 1985, 1988). However, more recent genetic analyses have not found evidence for such pleiotropy at the genomic level (Kukekova et al., 2011). Furthermore, Lord et al. (2020a) also point out that the starting point for the experiment was domestic farmed foxes and not wild ones. As such, all these characteristics were most likely already present in the population. Thus rather than representing genuine pleiotropy, these results more imply that these traits may be in linkage with loci for tameness, or that due to inbreeding and population substructure these traits became more prevalent in the tame-selected population.

What types of mutations cause the domestic phenotype?

Having seen how gene expression differences can lead to variation in a phenotype and their architecture in the genome, it remains to be seen what kind

of mutations actually cause these gene expression differences and hence the domestication phenotypes. The genome itself is usually made up of between 13k and 30k genes, but the actual size of the genome can vary tremendously between species. Some genomes are rather compact (e.g., the chicken is around 1.09 Gb), while others are larger (humans have ~3 Gb and mice ~3.4 Gb). The genes themselves are made up of exons (which are actively transcribed and often translated to form proteins) and introns (the regions between exons that are spliced out of the gene during transcription).

Promoters are regions at the start of a gene that help determine how the gene is regulated, while other enhancers and transcription factor binding sites can be present around the gene (commonly referred to as a cis effect—when the mutation is close to the gene it is affecting) or even much further away (trans effect) and also regulate the degree of expression. Therefore mutations can either affect the coding regions themselves (resulting in altered proteins, or even the absence of a particular protein), or the noncoding regions that regulate expression. Examples of coding-change mutations are generally more abundant, though this is ascertainment bias, due to the increased ease of identification of this type of mutation. This is due to the greater ease of showing that a protein is either truncated or altered after such a mutation, whereas for noncoding changes a multitude of candidates may be present in a region and the exact location is far harder to prove without transgenic evidence or similar advanced techniques. See Carroll et al. (2005) for an overview of genome organization and the different mutation effects.

Examples of coding changes affecting domestic animals include examples from coat coloration in chickens (insertion/deletion polymorphisms (Kerje et al., 2004)) and horses (missense mutations (Brunberg et al., 2006)), muscling in dogs (in this case a premature stop codon—(Mosher et al., 2007)) and cattle (Grobet et al., 1998). There are growing numbers of examples of cis mutations, whereby noncoding changes affect a trait. In domestic animals, examples exist for pig fatness (Van Laere et al., 2003), sheep muscling [the creation of a microRNA binding site (Clop et al., 2006)], color (Eriksson et al., 2008), and comb shape (Wright et al., 2009) in chickens, with more occurring all the time. Indirect evidence may be taken by the large numbers of gene expression variation seen between wild and domestic animals (reviewed earlier), with this more likely to occur through regulatory changes (though this may be harder to prove, with coding changes not detected in such screens).

Although to date the debate has been regarding which mutation types are more common, it is certain both these two types play very important parts in the expression of a phenotype. What is perhaps more interesting is whether the noncoding (cis or trans) mutations are qualitatively distinct in terms of their relevance to evolution and development (Wray, 2007). In principle, regulatory mutations may be more pliable for causing quantitative changes due to the nature of pleiotropy and codominance. For example, certain

phenotypes may be easier to modify using cis mutations than coding mutations. Given the relatively small numbers of genes present in the genome, a toolkit approach occurs, especially with developmental genes (Carroll et al., 2005), with genes used in a multitude of different circumstances.

Cis mutations can cause specific changes in a given tissue at a given time point, while expression in other tissues will be unaffected. For example, many of the genes identified in the previous studies (*IGF2* for pig fatness, myostatin in sheep, *BCDO2* in chickens, etc.) are all expressed in a wide variety of tissues at a wide variety of times. Therefore any changes in a coding region will lead to that effect being manifested in every tissue and time point that protein is expressed. In that way, such protein-coding changes may be far more pleiotropic, whereas regulatory mutations will allow more specific effects to be manifested without relying on potentially large trade-offs.

Furthermore, most regulatory mutations are codominant, whereas protein-coding changes are recessive (i.e., two copies are required to manifest an effect). This enables selection to act more efficiently on heterozygotes in the case of regulatory mutations (as there will be a difference between the heterozygotes and the two different homozygotes).

Copy number variation

Copy number variation (CNV) refers to the situation whereby a region contains one or more duplications (or indeed deletions) in one individual relative to other individuals. Technically, these regions are greater than 1 kb (1 kb = one thousand bases) and can be up to several megabases or even greater in length (Henrichsen et al., 2009), though more recently much smaller duplications are now being detected and classed as CNVs.

Initially, the analysis of such CNVs rose to prominence around 2006/2007 (Feuk et al., 2006), though now they are becoming more and more a part of standard analysis for the detection of the genetic basis of phenotypic diversity. In standard analysis, they are typically detected using a Competitive Genomic Hybridization Array (Pinkel et al., 1998), whereby the intensity of regions are compared between two different individuals, pools, or populations for example, and the intensity gives an indication of the degree of duplication. More recently, whole-genome resequencing can also be used for this. In this case, individuals have their entire transcriptome (all transcribed genes) sequenced using resequencing technology. The transcriptome is first amplified and then randomly broken into smaller segments. These segments then have the first (and in some cases last) parts "read," with these reads then aligned against the reference genome. This allows both a very accurate distinction of which precise allele is being expressed, and the relative number of reads for each given region can then be compared between multiple individuals or pools of individuals.

Such CNVs have been found to typically occur in regions of segmented duplication (SD—regions of DNA with almost identical sequence) and also in genes affecting immunity, sensory perception, and response to stimuli (Clop et al., 2012). There can even be large differences between individuals—for example, even within mouse inbred strains (thought to be largely isogenic—meaning essentially clones of one another), differences in CNVs occur. Domestic animals appear no different to the other species looked at, with SD regions and immune genes harboring the greatest numbers of CNVs, with such CNVs found in cattle, pigs, chickens, and dogs among others (Fadista et al., 2008; Liu et al., 2010; Wang et al., 2010; Clop et al., 2012).

The effects of CNVs can be extremely varied, as can their mechanisms. Confining ourselves to domestic animals (Clop et al., 2012), effects have been found on color in horses (premature graying (Pielberg et al., 2008)), goats (Fontanesi et al., 2009) and sheep (Norris and Whan, 2008) (both with a CNV in the *ASIP* gene), morphology in chickens [causing the classic pea comb variant (Wright et al., 2009)], and also late feathering in the same species (Elferink et al., 2008). In dogs, CNVs are related to the ridge mutation in ridgeback dogs (Hillbertz et al., 2007) and chondrodysplasia in a variety of small dog breeds (Parker et al., 2009). These examples are all discrete mutations. However, one study did find an association between production traits and CNVs in cattle (Seroussi et al., 2010), though in this case the exact mechanism has not been conclusively proven.

Moving away from domestic animals, other examples of correlations between complex diseases and CNVs have been identified (Zhang et al., 2009). Associations with neurocognitive defects (Brunetti-Pierri et al., 2008), autism (Sanders et al., 2011) and schizophrenia (Sebat et al., 2007), are of particular note in a behavioral setting. The actual mechanisms by which CNVs affect the expression of a trait can be extremely varied and range from the simple to the complex. At the most basic level, deletions (a form of CNV) can remove one or more exons from one or more genes (or indeed even entire genes), leading to large changes in the protein that is expressed. More subtly, dosage effects can be found, with the greater the number of copies of a gene, the greater the expression (especially if the gene promoter is also duplicated). Further mechanisms can be more complex still, with transcriptional sites that up- or downregulate a gene altered through dissociation between these sites and their promoters, alteration of chromatin structure, and the like [see Henrichsen et al. (2009) for a brief overview], though little is yet known about such regulation.

Mapping genes for behavior—top-down approaches

The mapping of genes for behavior is similar to mapping other quantitative traits, only with the exception that the trait is often harder to define and

measure reliably. The general methods either use a top-down or a bottom-up approach. Essentially, this refers to whether one starts at the phenotype and attempts to work down to the gene level, or whether one starts at a gene level and attempts to work up to the phenotype (Boake et al., 2002).

Pedigree studies and heritability analysis

Initially, this uses statistical analysis of pedigrees to demonstrate an actual heritable component to the trait of interest, through breeding designs, artificial selection, or the like. The requirements at this stage are only the behavioral phenotypes for all individuals and knowledge of their relatedness (the pedigree). Heritability studies can further dissect traits down to distinguish between broad sense and narrow sense heritability, with the genetic component further dissected down to additive and dominance components (additive, in this case, refers to those loci that give a cumulative effect, whereas dominance represents the interactions between alleles at the same locus) (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

Quantitative trait loci and association mapping

QTL analysis is nowadays a very well-established technique, which became increasingly popular in the 1990s (Lander and Botstein, 1989). In recent times, it has enjoyed something of a renaissance, first with the use of microsatellite markers, and now with single nucleotide polymorphism (SNP) markers and resequencing technology. These finally are enabling the step to gene discovery to be performed (previously very much a weakness with this approach).

At its most basic, this is the crossing of two distinct populations that differ for one or more traits of interest. As such, this technique is particularly amenable for domestic animals, where extremely large phenotypic differences frequently occur between wild and domestic populations (Andersson and Georges, 2004). The intercross individuals are then genotyped for multiple markers spread throughout the genome, with the genotype information correlated with the phenotypic data. This enables the identification of the number of loci affecting the traits that differ between the two populations, their effect size, and their genomic location. The loci can be defined as their additive and dominance components, while pleiotropy and epistasis (multiple genes interacting with one another) can also be identified. Two-way epistasis is the only realistic form of detectable epistasis, due to the massive increase in the number of tests performed (essentially an exponential increase).

As mentioned, the major issue is one of the resolutions of the detected loci (with very large confidence intervals generally the norm) and the sample sizes required for the detection of smaller effect loci (Beavis, 1998). Although this is an extremely flexible technique, the resolution of the

detected loci is limited by the number of recombinations present in the test cross (Darvasi, 1998; Lynch and Walsh, 1998). Typically, this means that the resolution of each QTL is rather low, and relatively few QTLs have been refined to the causative gene (often referred to as quantitative trait genes or quantitative trait nucleotides if the exact mutation is identified) though this is beginning to change.

Association mapping is a similar technique, but frequently uses a single outbred population and a high-density SNP analysis, to identify QTL. The difference with this technique is that as outbred or single large populations are used, many more recombinations are present and therefore the resolution is far greater. In contrast, however, many more markers are required per individual and population substructure can be an issue. Additionally, in such large populations, the genetic architecture tends to be very complicated (as far more polymorphisms and alleles are being considered than in a standard QTL cross that utilizes inbred populations/individuals and most commonly only two different populations). Results with association mapping in large populations in humans tend to show that very few QTL with large effects are present, and very little of the variance present can be explained by the loci that are discovered (Carlson et al., 2004). However, in the case of domestic animals, they possess many features that make them more amenable to this analysis (Goddard and Hayes, 2009). Firstly, the strong directional effects of domestication selection can mean that fewer markers are required as haplotype blocks are larger [for instance in the case of the dog (Karlsson et al., 2007)] and the genetic architecture is less complex than more outbred populations, in terms of the numbers of alleles present that affect the trait at any given QTL. This does have the drawback that only large regions are identified, however, when combined with multiple breeds very narrow regions can be identified. Jones et al. used this to tentatively map QTL for distinct breed characteristics, including pointing, herding, boldness, and trainability (Silva et al., 2009).

Selective sweep mapping

Linkage disequilibrium and the identification of selective sweeps is potentially an extremely powerful tool for the dissection of genetic architecture and shows great potential in domestic populations (Andersson and Georges, 2004). The basis of selective sweeps is that when a new mutation arises in a population and is then subjected to strong directional selection, not only it but the haplotype it arose in will go to fixation. The surrounding SNP markers will therefore "hitch-hike" alongside the mutation (Smith and Haigh, 1974).

Once fixation is reached, depending on the strength of selection and the time that has elapsed since fixation, this signature of selection will be slowly eroded. In the case of domestic populations, the strength of selection is often

extremely high, increasing the likelihood of such sweeps occurring, though this is also dependent on the genetic architecture of the trait (Pritchard and Di Rienzo, 2010). Such sweeps have been seen in the chicken, where around 50 40 kb regions were putatively identified as being under selection in domestic layer and broiler populations (Rubin et al., 2010), the dog [with blocks of around 106 kb in length (Goldstein et al., 2006)], and even the stickleback [sweep sizes between 20 kb and 90 kb in size (Makinen et al., 2008)]. This approach is particularly powerful when multiple domestic populations are analyzed, with shared regions of identity-by-descent identified, and has been used to locate both discrete mutations (e.g., the pea comb (Wright et al., 2009) and yellow skin (Eriksson et al., 2008) mutations in chickens) and mutations affecting quantitative traits, for example, pig fatness (Van Laere et al., 2003), cattle twinning rate (Meuwissen et al., 2002) and milk production (Riquet et al., 1999), and comb size in chickens (Johnsson et al., 2012, 2014). In the case of comb size in the chicken, a further study was performed looking at selective sweeps in a population of Hawaiian feral chickens (Gering et al., 2015; Johnsson et al., 2016b). Feralization is a process whereby domestic animals are either released or escape back into the wild, where natural and sexual selection forces can once again act on them (Gering et al., 2019a,b). In the case of the chicken, selective sweeps in feral birds were found to overlap with genes that had been shown to affect comb size in a wild × domestic intercross (Johnsson et al., 2016b), suggesting that these loci were undergoing active selection in the feral population analyzed.

Mapping genes for behavior—bottom-up approaches

With this approach, research starts at the gene level and is built up to the behavioral phenotype. In its purest form, gene knock-outs, knock-downs (whereby the expression of a gene is reduced using a variety of different techniques), and transgenics (the insertion of a novel gene into a genome) (Flint and Mott, 2001) can be used to direct the effects of individual genes, though one must still find which genes should be perturbed before analysis. Often such research therefore starts with a mutagenesis screen, whereby large numbers of mutant individuals are generated and then bulk screened for alterations to the behavior of interest (Nadeau and Frankel, 2000).

Such an approach has produced many of the early successes with behavioral gene identification, though these were restricted to the circadian rhythm in *Drosophila* (Tully, 1996; Sawyer et al., 1997), social aggregation in *C. elegans* (Coates and de Bono, 2002; de Bono et al., 2002), and larval foraging in *Drosophila* (de Belle and Sokolowksi, 1989; Sokolowski, 1998), so the relevance to domesticated animals may be lower, especially considering the size and generation times of many of the domestic animals under investigation.

Moving up, global gene expression analysis, traditionally through microarray analysis though more recently through RNA-seq (whole-genome

sequencing of the transcriptome) gives more precise measures of transcription (Wang et al., 2009). The issue with this approach is the amount of data generated and interpreted. For instance, what tissue does one look in and what time point? A particular gene may be limited in its expression to a very narrow window and even then relatively modest differences in expression may cause the phenotypic change. Additionally, if you find differentially expressed genes between populations, for example, many hundreds or even thousands can be identified, but determining the critically important genes from those that are due to downstream changes of such key regulators can be extremely complicated (Verdugo et al., 2010).

Merging both a top-down and a bottom-up approach, expression QTL (eQTL) analysis uses a combination of expression microarrays run on each individual from a standard QTL study (Gibson and Weir, 2005). In essence, the microarray gene expression values are used as phenotypes to be correlated with the genotypic marker information for each individual. The difference here is that the physical position of the gene is known, so what is mapped is the causative element leading to variation in the gene expression. Obviously, the same issue with tissue and time point choice is still present, but this does allow the correlation with a behavioral phenotype, which can be much more persuasive evidence of causative gene identification (Mehrabian et al., 2005).

Effects of specific mutations

Social aggregation in *C. elegans*

The identification of the actual mutations that lead to variation in behavior has been notoriously hard to identify, while none have been truly identified for domestication behavior in a domestic animal. Having said that, the lessons learned from the few examples identified in model organisms can give important insights into the type and effect such mutations will no doubt have.

Beginning with social aggregation, one of the classical examples for this actually comes from the nematode *C. elegans*. In the wild, there are two naturally occurring variants of worms feeding on *E. coli*—social and solitary foragers (Hodgkin and Doniach, 1997; de Bono and Bargmann, 1998). Greater than 50% of social foragers are found in groups, while less than 2% of solitary foragers are found in such states, irrespective of age and sex. The social foragers move more rapidly (de Bono and Bargmann, 1998) and accumulate at the richest food regions. However, social foragers do not aggregate in the absence of food (de Bono and Bargmann, 1998), implying ecological factors are also important to the manifestation of this behavior. Both morphs are associated with natural variation at the *npr-1* (neuropeptide receptor resemblance) locus, although several other loci may affect this behavior, albeit in a lesser manner. This locus was identified using a mutagenesis screen and encodes a neuropeptide receptor, similar to neuropeptide

Y receptors (G-proteins, regulating food consumption, anxiety, and pain in mammals). These similarities suggest *npr-1* has a neuropeptide ligand, however, no clear NPY (Neuro-Peptide Y) homolog has been found. Null mutations in *npr-1* transform solitary feeders into strong social feeders, with a specific single aminoacid change in *npr-1* also found in 17 natural isolates (de Bono and Bargmann, 1998).

The gene npr-1 regulates this aggregation through stimulating aversion to noxious stimuli (de Bono et al., 2002), utilizing specific neuronal pathways (the ASH and ADL neurons). If these neurons are ablated, the social aggregation response is severely decreased. Aggregation is regulated by food in *C. elegans* (de Bono and Bargmann, 1998) therefore food, in this case, is the aversive stimuli, not surprising given that soil bacteria can kill *C. elegans* (Marroquin et al., 2000). In mammals, NPY has also been shown to have sedative effects and decreases the sensitivity to nociceptive stimuli (Naveilham, 2001), showing a similar trend to the *C. elegans* model system with *npr-1* activity suppressing the behavior induced by noxious stimuli.

Foraging in *Drosophila* larvae and *Apis mellifera*

Another classic mutation affecting behavior, causing extreme effects and largely attributable to a single gene locus, is found in the *for* gene (Sokolowksi, 1980; de Belle and Sokolowksi, 1989). Individuals with what is termed the "rover" allele move greater distances while feeding than those homozygous for the "sitter" allele, with neither showing any variation in behavior in the absence of food (Pereira and Sokolowksi, 1993). Both of these behavioral morphs exist at appreciable frequencies in the wild (70% rover; 30% sitter) (Sokolowksi, 1980, 1982). The *for* gene itself is in fact the gene *dg2*, one of two cGMP (cyclic Guanosine MonoPhosphate)-dependent protein kinase genes (PKG) in *Drosophila*, with the cGMP signal transduction pathway implicated in foraging behavior in *D. melanogaster* (Osborne et al., 1997). PKGs function in naturally occurring variation in larval foraging behavior, with gene levels correlating with larval foraging behavior (Sokolowski, 1998). Sokolowski et al. (1997) used an empirical study comprising three separate base populations, each selected for either high or low population density. Those individuals in a low-density selection environment had shorter foraging path lengths, while those from high-density environments had longer path lengths.

The evolutionary benefits of these different phenotypes are therefore apparent in varying density environments. At high densities, larvae are required to navigate around other individuals to reach food patches, while under low-density conditions this locomotion behavior is an unnecessary waste of energy, with food being continuously distributed and in greater quantity. *For* is found to be expressed in a diverse set of tissue types, principally those involved in taste, olfaction, gut, and central brain function [closely paralleling rat PKG1 expression—(Kroner et al., 1996)] and thought

to be involved in the response to aversive stimuli. The association between social behavior and neurons that respond to aversive conditions in this instance, therefore, provides a mechanistic basis for these observations from behavioral ecology. Such adverse environmental conditions often provide the stimulus for group formation [for instance, antipredation behavior in fish (Krause and Ruxton, 2002)].

Pigmentation and behavior

As seen in ancient wall pictures and as obvious from recent selection experiments, loss of pigmentation seems to be one of the first and most conspicuous phenotypic changes occurring in most domesticated species (Clutton-Brock, 1999; Price, 2002). This leads to a rapid increase in the frequency of spotted and white individuals in the population. It is of course quite possible that the fast emergence of hypopigmented individuals is a result of directed human selection of rare, spontaneous mutations. For example, white and spotted individuals may have been easier to find and recognize and to distinguish from the wild ancestors, which would have been abundant around the early farm villages, and hence may have been perceived as more attractive. However, it is also possible that loss of pigmentation is related to tameness and reduced stress sensitivity, as we discussed earlier in the perspective of the Belyaev experiment on foxes. In this case, nonpigmented animals may have developed spontaneously and could have been favored during selection either because of their visual attractiveness or because of their tameness, or both.

Several studies of different species indicate a clear correlation between pigmentation on one hand and stress sensitivity or fearfulness on the other. For example, nonpigmented mink are generally easier to handle than their colored conspecifics (Trapezov et al., 2008) and in wild vertebrates, darker and more pigmented species and populations are generally more aggressive and more sexually active (Ducrest et al., 2008). Salmon selected for low cortisol response in a stress test have fewer pigmented skin spots than more stress-prone individuals (Kittilsen et al., 2009).

Skin and hair pigment comes in two different variants: black eumelanin and red phaeomelanin. The phenotypic expression of both is mediated by the melanocortin receptor, which in turn exists in five different variants, present in different tissues. The most important one for the expression of pigmentation is melanocortin receptor 1 (MC1R), a G-protein-coupled receptor in melanocytes. When bound to the agonist α- melanin stimulating hormone, the receptor causes a cascade of reactions leading to the deposition of black eumelanin in the melanosomes; whereas, the receptor antagonist, the Agouti signaling protein (ASIP) leads to deposition of phaeomelanin instead.

The key to how pigmentation may act pleiotropic to behavior and stress sensitivity lies in the fact that the melanocortin receptors are spread in many different tissues and that their ligands cause a variety of effects. A mutation

in the receptor gene may thus inhibit or modify pigment expression, while simultaneously altering the stress response system. This is further supported by observations that cortisol levels in dog hairs vary systematically with pigmentation: phaeomelanistic (red) hairs have higher concentrations than eumelanistic (black) while banded hairs (agouti) are intermediate (Bennett and Hayssen, 2012). Furthermore, in rodents, agouti-colored animals are more aggressive and less tractable than the nonagouti conspecifics (Hayssen, 1997; Cottle and Price, 1987).

Fang and coworkers studied mutations in *MC1R* of pigs from China and Europe and observed nine different protein-changing modifications in the gene in different populations (Fang et al., 2009). This is a striking difference to the case in the ancestor, the wild boar, where all observed mutations were silent (i.e., synonymous substitutions). The authors conclude that pigmentation in the wild ancestor is under strong purifying selection, whereas different phenotypically active mutations have been selected by different human populations. They argue that this shows that color variation during domestication is a result of direct selection for attractive color phenotypes, rather than a side-effect of selection for tameness. However, it is also possible that different mutations in pigmentation-related genes, such as *MC1R*, may cause similar pleiotropic effects on behavior. Behavioral differences between different pig mutants have not been studied, so it remains a possibility that selection has originally been on tameness rather than on color. As mentioned earlier, the two explanations are not mutually exclusive.

The phenotypic expression of pigmentation is of course also dependent on further downstream genetic processes, which may be related to behavior in different ways. In a large-scale QTL study of a cross between domesticated White Leghorn chickens and ancestral Red Junglefowl, it was observed that the risk of being exposed to feather pecking, a detrimental behavior disorder, was related to a mutation in the *PMEL17*-gene (Keeling et al., 2004). The same mutation was identified as the causative mutation in the Dominant White-locus and causes a lack of all melanin expression in feathers and skin (Kerje et al., 2004). The mutation protects against being exposed to feather pecking, and pigmented birds are more vulnerable to becoming victims when they are relatively common.

Follow-up studies showed that the specific mutation, a nine base-pair insertion in the transmembrane region, was related to differences in social and exploratory behavior, also when the birds were raised without the possibility to interact physically with conspecifics (Karlsson et al., 2011a,b). In general, pigmented birds were more aggressive and explorative. This shows a clear pleiotropic effect on behavior of a specific pigment-related mutation, but it remains unclear which mechanisms may be involved. Some hypotheses may, however, be discussed.

PMEL17 is a melanocyte-specific protein, which is not directly involved in the synthesis of melanin but rather affects the maturation of melanosomes,

the melanocytic vesicles where melanin is deposited. The protein forms amyloid fibers on the inside of the melanosome membranes, and these serve as the substate where melanin particles fasten (Yasumoto et al., 2004). The mutation inhibits the formation of a transmembrane region, and the further development of the necessary amyloid fibers, and thus leaves the melanosomes without any substrate for melanin deposition.

Melanin is formed from tyrosine and DOPA (dihydroxyphenylalanine) as precursors. DOPA is also a precursor for the synthesis of the potent neurotransmitter dopamine and the catecholamines norepinephrine and epinephrine. As a hypothesis, Nätt and coworkers suggested that when melanin cannot be deposited, its synthesis may be downregulated, and the concentration of DOPA may consequently increase (Nätt et al., 2007). This could in turn make more DOPA available for the dopamine synthesis pathway. Although this mechanism is highly speculative, the fact that there is a close biochemical connection between melanin synthesis and behaviorally important pathways makes a connection between pigmentation and stress responsiveness quite logical.

In cats and dogs, there is a huge literature on the genetics of pigmentation, mostly based on between-breed comparisons. Some of these studies imply a close connection between pigmentation and behavior (Kim et al., 2010; Stelow et al., 2016). As domestication is mainly associated with loss of pigmentation, a recent study used a Genome Wide Association Study (GWAS) to map genes associated with the degree of whiteness in a large population of laboratory beagles (Nord and Jensen, 2021). There was a close association with two loci, containing the well-known pigmentation-related genes *MC1R* and *MITF*, suggesting that pigmentation loss is largely a function of regulatory variation related to these.

Neurotransmitters and social behavior

Among the more prominent behavior changes occurring as a response to domestication is the modified social behavior. Whereas the wild ancestors of our domesticates usually live in groups with very little flexibility concerning size, dynamics, and home ranges, most domestic species tolerate very variable group sizes and a high degree of dynamic changes in group composition. For example, wolves live in packs that rarely exceed 15−20 members, and where all individuals mostly are closely related, whereas free-ranging dogs form variable sized groups of up to dozens of dogs where membership of a group may change from one week to the next (Boitani and Ciucci, 1995); Wild boars form tight family groups with little exchange of individuals, while domestic pigs tolerate being regrouped at regular intervals during rearing (Gonyou, 2001); Red Junglefowl live in small harem groups with strict territorial boundaries, while chickens under commercial conditions can cope with thousands of other birds in a limited space (Mench and Keeling,

2001). Mutations affecting sociality and social tolerance must have been important selection targets during domestication.

During recent times, there has been a growing interest in the role of neuropeptides, primarily vasopressin and oxytocin, in shaping the social behavior of an individual (Donaldson and Young, 2008). While the actual hormones are evolutionary well conserved, variations in the expression of their receptors have been experimentally connected to variations in social behavior both between and within species. For example, the expression of the vasopressin receptor gene *AVPR1a* is highly correlated with the degree of gregariousness across different finch species and with the degree of mate attachment in voles (Donaldson and Young, 2008). The expression differences appear to be largely connected to variations in the length of a highly polymorphic microsatellite (a stretch of repetitive DNA sequences) located at the 5′-flanking region of the gene. It seems likely that the length of this microsatellite may affect the transcription level of the gene. Correlations between the length of the microsatellite allele and sociality have been demonstrated in birds, voles, and humans.

The other of the two closely related nine-aminoacid neuropeptides, oxytocin, has received a large interest for its role in social bonding and nursing and nurturing in humans. It exerts basically the same effects across different mammalian species, where increased levels of circulation are associated with a stronger bonding and nurturing tendency (Donaldson and Young, 2008). It appears as oxytocin mainly exerts its effects on females while vasopressin chiefly is the male effective hormone.

Selection for favorable variants of the receptor genes of these neuropeptides would hypothetically be a plausible pathway for causing the domesticated social behavior, which seems to be characterized by increased social tolerance and an increased ability to bond with humans. However, there has been little direct research into this possibility. Meanwhile, some suggestive observations lend a bit of support to the hypothesis.

On a large scale, QTL analysis of a cross between Red Junglefowl and domesticated White Leghorn layers, a major QTL affecting growth, and several other domestication-related traits was discovered on chromosome 1 (Schutz et al., 2002; Kerje et al., 2003). The QTL was named Growth 1, in recognition of its immense effect on growth rates—the genotype at this locus explains more than 20% of the difference in adult body weight between the breeds. In addition, Growth 1 also affects several other domestication-related traits, such as egg production and fearfulness, as measured in open field tests.

A closer analysis of the gene content in the region showed that *AVPR1a* is located centrally in the QTL. Also, another vasopressin receptor *AVPR2*, as well as the oxytocin gene, are well within the confidence interval of Growth 1. A plausible hypothesis would therefore be that social behavior could be modified by these loci as a correlated response to selection for increased growth.

Wirén and colleagues bred birds from an advanced intercross line between Red Junglefowl and White Leghorns to be homozygous for alternative alleles at a microsatellite marker in Growth 1, closely linked to *AVPR1a* (Wiren and Jensen, 2011). These birds, and birds from the pure lines, were tested in different social setups. Red Junglefowl, as expected, were more aggressive toward intruders and less inclined to interact with strangers. In the intercross birds, the difference in genotype on the selected marker locus close to *AVPR1a* caused the birds to show breed typical differences in males, but not females, indicating that the gene may be a major player in causing the domestication induced differences in social behavior. As further support to this, the gene is differentially expressed in the part of the brain containing the ventral pallium when comparing Red Junglefowl and White Leghorn males (Wiren, 2011).

Of course, these studies are purely based on correlations, and the effects could just as well be caused by any other linked gene in the same region. However, it is intriguing that several socially potent neuropeptide genes are located in the same small genomic region. It remains to be studied which mutations may underlie the different expression rates, and it is also as yet uncertain to what degree this locus is involved in shaping social behavior in chickens.

In dogs, variants in the regulatory region of the oxytocin receptor gene have been implicated to cause variation in social behavior both toward other dogs and toward humans (Kis et al., 2014; Jensen et al., 2016). The variation in receptor genotype has been found to affect the behavioral responses to intranasal oxytocin administration. Dogs with one genetic variant increased their interactions with humans in a problem-solving task when oxytocin was administered, whereas dogs with another variant showed the opposite response (Persson et al., 2017).

Different means to same ends

The traditional domesticated animals have a history of thousands of years of living with humans, making it very difficult to pinpoint early genetic responses to the altered selection. However, studies of recently added members to the domesticated fauna may be interesting in this respect. Many fish species have been domesticated only in the last hundred or so years, while at the same time being under similar selection pressures as other domesticates. For example, they are bred to grow faster than their ancestors in an environment where food is abundant and predation is virtually absent.

Tymchuk and colleagues studied gene expression profiles in domesticated rainbow trout and their wild conspecifics in different tissues and further compared this to other domesticated salmonids (Tymchuk et al., 2009). They found that transcription profiles could differ substantially between different species, but taken together they target the same physiological end-point.

The common denominator appears to be to affect the growth hormone gene and the closely associated *IGF1*. This suggests that although the phenotypic target is the same, the genetic networks are sufficiently complex and flexible to cause a similar phenotypic effect by means of different pathways. This would be an important argument against the previously mentioned hypothesis that domestication is a single-gene event.

The fish studies also demonstrate the existence of important pleiotropic effects, which conversely adds some support to the idea that broad phenotypic alterations can be caused by the selection of a single trait. For example, Johnsson et al. (1996) studied brown trout (*Salmo trutta*) that were either hatchery raised or injected with growth hormone. Both treatments caused a similar increase in growth rate. Irrespective of whether growth had been increased by selective breeding or by hormonal manipulations, the correlated behavioral responses were very similar. Both types of fish had a reduced antipredator behavior, explained by the authors as a response to a reallocation of resources from vigilance and alertness to growth. Similarly, Wright et al. found correlations between growth and fear-avoidance behavior in an intercross population between wild and laboratory zebrafish (Wright et al., 2006c).

Epigenetics

So far, we have discussed how different mutations and variants in DNA sequence may be involved in causing the phenotypes associated with domestication. According to current genetic and evolutionary theories, spontaneous and randomly occurring mutations are the only substrate for natural and artificial selection. As we have seen, many of the identified mutations are mainly regulatory and do not affect protein structures. So the strongest driving forces behind evolutionary change appear to be related to the timing of expression of protein-coding genes, or in other words, how the genome is orchestrated during ontogeny.

This has sparked an increased interest in understanding the mechanisms involved in gene regulation. Some of these mechanisms are purely genetic in their nature, as explained above when we discussed eQTL studies, in which case the expression profile of a particular gene is contingent on the DNA sequence on another locus. However, a substantial part of the expression variation is due to chemical modifications, which do not affect or relate directly to the DNA sequence. These are the factors we refer to as epigenetic (Richards, 2006), and they represent a recently discovered and as yet poorly understood bridge between the environment and the genome.

At the molecular level, mainly two chemical changes have attracted research so far. The first consists of a modification of cytosine bases, where a methyl group is added to the fifth carbon, changing C to 5mC. Most methylation of cytosine occurs when this nitrogen base is positioned next to a

guanine base, a so-called CpG-position, and throughout the genome, repetitive sequences of this combination occur mainly in regulatory regions, in so-called CpG-islands. Increased methylation is usually associated with decreased expression of the gene.

The second important group of epigenetic modification affects histones, the proteins responsible for packing DNA in the nucleus. The histone tails may receive additions of, for example, methyl- or acetyl-groups, which will change the density with which DNA is packed. Consequently, the more densely packed the DNA, the less transcription of the genes in the region. It is important to remember that although both DNA-methylation and histone modifications are important regulators of genome activity, they do not affect the DNA sequence itself. A novel term to capture the status of the genome with respect to its epigenetic modifications is "epigenome."

A large proportion of the epigenetic variation is constitutional—it varies vastly between cell types and organs, but within a particular cell type, variation is much smaller between individuals (Richards, 2006). This constitutional variation depends on genetic differences, either in the promoter regions or in trans-located loci. However, some of the epigenetic variations are dynamic and modifiable, so loci involved in this may be highly responsive to environmentally mediated input—hence, the epigenome can be thought of as a bridge between nature and nurture.

Epigenetic changes and long-term behavioral modifications

In a pioneering series of experiments, Meaney and coworkers studied the long-term effects of early experience on later behavior and particularly investigated the epigenetic mechanisms involved (Weaver et al., 2004). In a cross-fostering experimental design, rats were raised either by attentive and caring mothers (which licked their pups a lot and performed much arched-back nursing) or by less careful females. Offspring of the more caring mothers were more resistant to restraint stress when adult and displayed a more attentive and careful maternal behavior when they themselves gave birth. This was found to be due to an induced difference in methylation status of a promoter region of the glucocorticoid receptor gene (*GR*), particularly the binding site associated with the transcription factor *NGFI-A*, also known as *EGR1*. The methylation differences between differently nurtured rats also caused histone modifications in the same region, leading to a modified expression of *GR*.

This experiment was one of the first clearly demonstrating that experiences gained during ontogeny can alter the epigenetic state of the genome and subsequently give rise to behavioral differences. A number of other experiments have demonstrated similar effects after this. For example, maternal care has been shown to affect methylation states of estrogen receptor alpha in rodents (Champagne and Curley, 2008), and epigenetic factors

affect cognitive and behavioral traits in a variety of species, including humans (Gräff and Mansuy, 2008).

Stress plays a crucial role in the interrelationship between environmental factors and epigenetic modifications. Although different definitions of stress are ubiquitous, there is a general consensus that steroid hormones, such as cortisol (or corticosterone), testosterone, and estrogens, are usually elevated under conditions perceived by an individual as stressful. Steroids act as DNA transcription modifiers when the steroid-receptor complex binds with binding proteins to specific DNA regions, and this provides an open gate from stress experiences to modifications of gene expression (Murgatroyd et al., 2009).

One of the few experiments to actually measure quantitative variation in behavior and methylation to check for correlations was performed using an intercross population of wild × domestic chickens by Höglund et al. (2020a). Although relatively few behavioral traits were found to possess a significant correlation with methylation (as measured throughout the genome using a sequencing-based approach), around half of the traits that did show a correlation were related to the regulation of intraindividual variation (Höglund et al., 2020a). Intraindividual variation describes how inherently variable or predictable an individual is and can be considered a trait in and of itself (Dingemanse et al., 2010). In the case of the chicken, domestic chickens are more variable than their wild counterparts at a genetic level (Henriksen et al., 2020). This could imply that methylation is linked with the regulation of variability in behavior, being a way of increasing or decreasing behavioral variability.

Epigenetic changes in domestication

Long-term effects of epigenetic modifications can also be transferred across generations, by changes in the germ-line epigenomes. Skinner and coworkers exposed rats to the environmental pollutant vinclozolin, which caused a dramatic effect on the methylation states and expression levels of many genes, and also affected their stress responses and anxiety-related behavior (Skinner et al., 2008). Both the epigenetic effects and the behavioral modifications were clearly measurable in the third, untreated offspring generation. This shows that epigenetic effects may alter phenotypes in ways similar to those of DNA mutations (although perhaps often in a reversible manner) and offers a hypothetical novel route for rapid evolutionary changes in response to, for example, environmental stress. A broad review of studies supporting this has been provided by Jablonka (2009).

As domestication is an example of precisely such a rapid evolutionary process, causing wide phenotypic modifications in a short time, it is quite likely that epigenetic mechanisms may play an important role here. Lindqvist and coworkers studied this in chickens, by exposing groups of ancestral Red

Junglefowl and domesticated White Leghorns to chronic stress during rearing (using unpredictable light scheme as stressor), and measured the outcome on a spatial learning task. In both breeds, stressed birds showed a reduced learning ability compared to control groups (Lindqvist et al., 2007). However, only in domesticated chickens was this decreased learning mirrored in the offspring. Using microarray analysis, the authors found that stress-induced brain gene expression differences were correlated across generations in domesticates, but again, not in Red Junglefowl. Hypothetically, it was suggested that domestication could have selected animals with an increased capacity to respond epigenetically to environmental stress and to transfer these responses to the next generation. In effect, the suggestion is that domestication may have selected animals with higher evolvability (Carter et al., 2005), which would have speeded up the pace of evolution considerably.

This suggestion is as yet hypothetical and speculative, but it has received certain experimental support lately. Nätt and colleagues used microarrays to analyze differential gene expression in the hypothalamus regions of a small number of families of Red Junglefowl and White Leghorn, and in addition studied the methylation differences in the promoter regions of more than 3000 of the same genes (Natt et al., 2012). The families were selected based on divergent results in a fear test, so within each breed, there were highly fearful and less fearful individuals. As in the previous studies by Lindqvist et al., fearfulness was significantly correlated between generations in White Leghorns, but not in Red Junglefowl, again indicating that the ability for transgenerational effects may have been favored during domestication.

Comparing the breeds, close to 300 genes were differentially expressed in the parents, and more than 1600 in the offspring. Gene Ontology analysis showed that genes belonging to pathways associated with stress responses, memory consolidations, neural differentiation, and reproduction were enriched among the differentially expressed ones. Looking at promoter regions, over 200 of the 3600 selected genes were differentially methylated in parents, and more than 800 in the offspring. Among genes carrying differential methylation, 79% were hypermethylated in White Leghorns, indicating that this breed has accumulated methylations during domestication. This suggests that selection for favorable epigenetic variation may have been an important aspect of domestication.

A particularly interesting aspect of the experiment is the fact that differential gene expression profiles, as well as methylation differences, were reliably inherited by the offspring, so there was a high transgenerational stability in the epigenetic effects. This further supports the importance of epigenetic selection as a driver of phenotypic changes during domestication. The last piece of evidence along this line from the same study is the observations that differentially methylated loci were significantly more common in previously identified selective sweep regions associated with domestication of layer breeds. This strongly suggests that a significant proportion of the

observed differential methylations may have been subject to directed selection.

One of the limitations of the earlier evidence is that while it is possible to show methylation differences between wild and domestic populations, this does not necessarily mean that the observed methylation differences are actually modifying gene expression. Evidence for the genetic basis of stable DNA methylation status and the role of methylation in domestication, and importantly evidence that such methylation can modify gene expression, comes from an intercross experiment using the chicken (Höglund et al., 2020a). By combining an intercross between wild and domestic chickens with both transcriptomic profiling of the hypothalamus and methylation sequencing using an antibody approach from the same tissue in the same animals, Höglund et al. (2020a) were able to demonstrate by using causality analysis how methylation and gene expression were linked in this intercross. Significant evidence existed to link gene expression and methylation changes, half of which showed methylation was causing the alteration in gene expression but in the remaining cases the reverse was true (i.e., gene expression was modifying methylation).

The study also found that stable epigenetic changes not only exist between wild and domestic chickens, but also that many of these epigenetic changes have a genetic basis. Using these data in a QTL framework, Höglund et al. could not only identify specific regions that were associated with either an increase or a decrease in methylation according to genotype, but also found that domestication had in general caused a decrease in methylation in long continuous stretches of the genome. Similarly, domestication "hotspots" were also identified that regulated methylation over a large number of genomic regions throughout the genome. This experiment demonstrates that stable differences in methylation exist between wild and domestic chickens, with these long-standing differences likely linked to genomic changes at CpG sites. In at least some of these cases, these stable epigenetic changes were also then leading to alterations in gene expression, as well as the reverse.

In another experiment, ancestral Red Junglefowl were selected for reduced fear of humans, as a way to mimic the early phases of domestication. Within a few generations, gene expression, as well as DNA-methylation in different brain regions, differed significantly between the selection lines, implicating that epigenetic variation is highly responsive to selection and thus may have been important for the rapid evolution during domestication (Bélteky et al., 2017, 2018).

Closing remarks

We are in the middle of one of the most dynamic developmental phases in biology ever experienced. Full genome sequencing can now be done at a low price and in a very short time, and epigenetic screening is developing at an

unprecedented speed. Hence, we can foresee a rapid increase in knowledge about how genetic variation is related to phenotypic differences. This will of course also be the case for animal behavior.

The challenge faced by scientists will be to provide reliable phenotypic measurements. Among all possible phenotypes, behavior stands out as particularly difficult. It is the first and fastest way in which an animal will react to any stimulus, and will therefore vary with almost any environmental factor (unlike, for example, color or size). The obvious solution to this is to standardize behavioral tests as much as possible, and this introduces the risk of distancing the recordings more and more from natural conditions. This is a highly sensitive balance, and it will take a lot of scientific skills from the researchers of the future.

The ultimate goal of the research will be to find the causative genetic polymorphisms responsible for domesticated behavior. This will be of enormous importance to anyone interested in the biology and welfare of domesticated animals, but equally so for those mainly concerned with the mechanisms of evolution.

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Chapter 3

How studying interactions between animal emotions, cognition, and personality can contribute to improve farm animal welfare

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ABSTRACT
This chapter provides an overview of the current behavioral and cognitive aspects of emotions in animals and explores the impacts of emotional experiences on the animal's adaptation to its current challenging circumstances. There is evidence that animal welfare results from the animal's perception of its environment and its background. The chapter is structured in four complementary sections. The first one addresses the nature of emotions that the animals can feel which is validated from commonalties in physiological and behavioral responses to dangers across and within species. The second section presents advanced features of the relationships between cognition and emotions originally studied in humans, which are now developed in animals to better access their affective states. The third section is devoted to the relevance of the personality concept, as resulting from both genetics and developmental experience, for assessing animal individuality in emotional behaviors and stress. The last section explores some approaches that can alleviate fear and induce positive affective states, with the potential to mitigate detrimental stress-induced effects on the welfare and health status by eliciting positive emotions in animals.

Preface to "How studying interactions between animal emotions, cognition, and personality can contribute to improve farm animal welfare" by Dr. Temple Grandin

It was important to reprint this chapter from the second edition because Alaine Boissey was one of the first scientists to introduce the concept of both emotions and personality into the farm animal literature. Some of his

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earliest work explained the importance of understanding how fear affects animal behavior. He explained that fear responses can sometimes appear contradictory. An animal can respond by (1) attacking, (2) hiding or escaping, or (3) passive avoidance (immobility). For the sake of simplicity, I prefer to call these fear-based behaviors fight, flee, or freeze. In this chapter, Boissey also recognized that an animal's personality is an individual difference in behavior that is "consistent over time and across situations." Fear is probably only one component of personality. Most of Boissey's work has been in farm animals but it is also relevant to wild animals. Homberger et al. (2021), who works with wild birds, stated that "Personality, i.e. Consistent individual Differences in Behavior." Unfortunately, as he is a wildlife researcher, he was not aware of Boissey's farm animal work. This is why it is important in science to read literature in other areas of behavior.

Recent research that was conducted after the second edition was published in 2014 revealed that genetic factors influenced two really important personality traits in dogs and horses. The animals became more social and less fearful. Variants in the structure of GTF21 and GTF2IRD1 genes are associated with friendliness and hypersociality in both domestic dogs and people with William−Beuren syndrome (Toth et al., 2018; Vonholdt et al., 2017). People with this trait are extremely friendly and have an intellectual disability. Toth et al. (2018) explain that sociality can vary from social deficits in autism to being highly social. It is likely that these genes are highly conserved in both people and dogs.

Another recent study of ancient DNA may explain the domestication of the horse. Librado et al. (2021) discovered that an increase in a gene called ZFPMI made horses more docile and reduced anxiety and fear because it increased serotonin in the brain. After this genetic change occurred, the use of horses rapidly developed. Mutant mice that were bred to be deficient in ZFPMH had stronger fear memories of places where aversive events occurred (Tikker et al., 2020). Giving these mice the drug fluoxetine (Prozac) reduced anxious behavior. This drug is very effective for reducing anxiety in people. These two studies both show that small changes in the genetic code that reduced fear and increased social motivation facilitated the domestication of animals. It needs to be made clear that both genetic factors and the environment will shape behavior, but two small genetic changes were associated with the greater use of horses. Change in another gene called GSDMC was associated with reduced back pain. The end result was a calmer horse that was less likely to have back pain from riding or pulling a chariot at a gallop.

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Introduction

The welfare of animals used for food production is a major concern for a society that stems from the recognition that animals are not only reactive to their environments but also sentient. Since the Brambell Committee (1965), it is clearly stated that animal welfare embraces both physical and mental well-being. Consideration of the mental well-being of animals implies that animals have emotional capacities, such that they attempt to minimize negative emotions (e.g., fear and frustration) and to seek positive emotions (e.g., pleasure and joy) (Boissy et al., 2007b; Dawkins, 1990; Duncan, 1996). Nevertheless, while it is now well accepted that animals express emotional reactions, there is still a persistent belief that we can do no more than observe how the animals behave and that we could never know how they feel. In addition, while the concept of welfare is based on a balance between negative and positive experiences (Dawkins, 1990; Duncan, 1996; Mellor, 2012; Spruijt et al., 2001), current research in animal welfare is mainly focused on identifying and preventing negative animal emotions and bad welfare. However, preventing negative welfare in animals is not the same as providing them with opportunities to experience positive emotions and positive welfare (Boissy et al., 2007b).

In addition to emotions, which are by definition fleeting, welfare implies more persistent affective states that influence the way in which the individual reacts to his environment (Lazarus, 1991). Repeated exposure to negative events is known to induce long-term stress-related responses such as depression-like behaviors. For instance, chronic social stress induces behavioral changes, such as decrease in locomotor activity, self- and allogrooming, feeding behaviors (both appetitive and consummatory components), and physiological changes, such as altered circadian rhythm, body temperature, or body weight in various animal species (Boissy et al., 2001; Carere et al., 2001; Coutellier et al., 2007; Veenema et al., 2003). Nevertheless, as noted by Dawkins (2001) and Dantzer (2002), research on

stress has generally been limited to indicators of stress and has not linked these indicators to the existence of long-lasting affective states. Therefore it is essential to move beyond the simple description of the emotions/stress-related reactions in farm animals towards a scientific approach of their own emotional experiences (DéSiré et al., 2002; Mendl and Paul, 2004). Recently, attention has been paid to animal cognition and appraisal as a promising approach to scientifically assess sentience in animals (Boissy et al., 2007a; Paul et al., 2005).

The main objective of this chapter is to highlight the emotions and the longer-lasting affective states of an animal, and the individual characteristics by which its emotions are influenced. This chapter follows four complementary threads. Firstly, we review emotions defined as resulting from their perception of their own environment, and we discuss the difficulties involved in the assessment of fear and anxiety, the most commonly studied emotions in farm animals, and the need for better validation of the experimental approaches. Secondly, we review the interactions between emotions and cognition and present the relevance of taking into account the cognitive abilities of animals to better approach their emotions and long-lasting affective states. Thirdly, we discuss the relevance of the personality concept, as resulting from both genetics and developmental experience, for better embracing the animal's individuality in emotional behaviors and stress. Finally, we explore positive emotions in animals and their interests not only to alleviate fear but also to induce long-lasting positive states close to the welfare concept and to attempt to mitigate detrimental stress-induced effects on the welfare and health status.

Do animals feel emotions?

What is the nature of animal sentience? What is an emotion? What is stress?

There is now a consensus in the scientific community that most animals that we use for our own purposes, whether for food, for work, or for animal experimentation, are acknowledged as sentient beings (Dawkins, 2001). Sentient animals are those who experience emotions associated with pleasure and suffering and who are motivated to promote their evolutionary fitness not as part of a well-planned, long-term strategy to ensure the well-being of future generations but through the simpler, but no less intense, need to feel good about themselves (Webster 2005).

This notion was included in the European Union Treaty of Amsterdam (European Union, 1997) highlighting the fact that because animals are sentient, their welfare matters (Duncan, 1993; Veissier and Boissy, 2007). This requires recognition that animals have emotional capacities and that they

attempt to minimize exposure to situations eliciting negative emotions such as fear, frustration, distress, and anxiety and seek situations eliciting positive ones such as pleasure and joy (Dawkins, 1990; Duncan, 1996; Spruijt et al., 2001). Improving our understanding of the range and depth of emotions that animals can experience is essential to safeguard and to improve their welfare. One of the key factors explaining the evolutionary success of emotions is that they would favor adaptive cognition and action. Emotions refer to processes that are likely to have evolved from basic mechanisms that gave the animals the ability to avoid harm/punishment or to seek valuable resources/rewards (Panksepp, 1994; Paul et al., 2005). Animals should thus be capable of assigning affective values to their environment.

Although there is no single general definition, an emotion can be defined as an intense but short-lived affective response to an event associated with specific body changes (Dantzer, 1988). An emotion is classically described by a subjective component, which is, strictly speaking, the emotional experience, and two expressive components, one motor (e.g., facial expressions, movements) and the other physiological (e.g., cardiac and cortisol reactions) (Dantzer, 1988). Emotions differ from sensations that are simply physical consequences of exposure to particular stimuli (e.g., heat, pressure) and from feelings that only designate internal states with no specific reference to external reactions. The emotional experience of animals is inferred from the behavioral and/or physiological components. There has been a growing interest in the study of emotions in animals over the last few decades, resulting in the emergence of a discipline referred to as Affective Neuroscience (Panksepp, 1998). Scientists have made huge progress in understanding how animals perceive their environment and the feelings prompted by this perception. First, in the 1970s, it became clear that the stress response, which was initially considered as a physiological concept (Selye, 1936), is triggered by psychological factors: it is the animal's representation of an event rather than the event itself that determines its stress reactions. This was inferred from studies on fasting monkeys who were either separated from or in the presence of normally fed counterparts (Mason, 1971). When separated from their normally fed counterparts, fastened animals were not aware of the threat imposed on their body and were not stressed, while they showed signs of stress in the presence of normally fed animals. Mason concluded that the nonspecificity of stress responses, reported earlier by Selye, was due to the common emotion—felt by the animal—that triggers stress responses.

It is now well known that stress responses vary in form depending on how the individual perceives the situation and its coping possibilities, such as whether the animal engages in a passive response (e.g., immobility) or an active one (e.g., fight or flight) (Dantzer and Mormède, 1983; Veissier and Boissy, 2007). Likewise, it became clear that the ability to predict the occurrence of a stressful event—painful or not—and to control the termination of

that event also affects stress responses (Weiss, 1972). This prompted scientists to suggest that animal welfare was closely linked to cognitive processes such as an awareness of some internal state (being hungry, being diseased, ...), expectancies about the environment (which help animals to detect whether something is absent or not), and the ability to predict or control the animal's environment (Duncan and Petherick, 1989; Wiepkema, 1987). From an adaptation perspective, the ability to perceive its own emotions enables the animal to detect and assess a discrepancy between its requirements and environmental conditions and the efficiency of its subsequent action to regain homeostasis. In addition, subjective emotional experiences represent motivational urges or drives that are predominantly negative and include breathlessness, thirst, hunger, and pain (Mellor, 2012). Therefore to illustrate the variety of approaches in emotion studies, we focus the following part on fear and anxiety because they are the most studied and the potentially most damaging of the emotions (Jones and Boissy, 2010).

Fear and anxiety

Fear is generally defined as a response to the perception of actual danger, whereas anxiety is regarded as the reaction to a potential threat (Boissy, 1998). Fear-related reactions are characterized by physiological and behavioral components that prepare the animal to deal with the danger. Such defensive reactions promote fitness: an animal's life expectancy is obviously increased if it can avoid sources of danger such as predators (Panksepp and Burgdorf, 2003). Although captive animals have few natural predators, the behavioral and physiological emotional responses persist (Dwyer, 2004). Moreover, domestic animals reared on ranges may still experience severe predation by wild animals or dogs (Asheim and Mysterud, 2005). Farm animals also show predator-avoidance reactions to human contact, even though reduced fear of human beings has been a major component of domestication (e.g., in herbivores: Price, 1984). Routine management procedures such as shearing, castration, tail docking, beak trimming, dehorning, vaccination, harvesting, herding, and transportation also cause fear and distress in cattle, sheep, and poultry (Gentle et al., 1990; Hargreaves and Hutson, 1990; Manteca et al., 2009; Wohlt et al., 1994). In addition, intense fear can cause chronic stress that may compromise the expression of fundamental behaviors (social, sexual, parental) and reduce productivity and product quality in cattle, sheep, pigs, and poultry (Bouissou et al., 2001; Faure and Jones, 2004; Fisher and Matthews, 2001; Hemsworth and Coleman, 1998).

Diversity of fear-eliciting events

According to Gray (1987), the fear-eliciting properties of an event reflect its general characteristics, for example, novelty, movement, intensity, duration,

suddenness, or proximity. Fear may also be elicited by specific stimuli, such as height and darkness or bright light, in relation to the evolutionary history of the species (ancestral fears). Additionally, a stimulus can elicit fear through association with previous experience of another fear-eliciting event (conditioned fear).

In modern farming systems, animals may be exposed to a large variety of potentially stressful events from hatching or weaning through to puberty and adulthood. These include social mixing, transport, transfer from a rearing or nursing environment to a growing one, dietary change, new social partners, exposure to different stockpersons, and harvesting. Gregariousness is common to most domestic animals (Keeling and Gonyou, 2001) and the variety of social stimuli that accompany social cohesion and structure may elicit or modulate fear reactions. Social signals may represent particular cases of the types of fear-eliciting stimulation mentioned earlier. Some social signals are characterized by their unfamiliarity, for example, the novelty of the neonate that affects maternal behavior in primiparous females (Poindron et al., 1984), alarm calls can spontaneously elicit fear (Boissy et al., 1998) and certain social odors can reduce fear and distress in the recipients (Madec, 2008). Some fear-eliciting social signals, for example threatening behaviors, may also be acquired (Bouissou et al., 2001). Under farming conditions, animals are frequently exposed to changes in their social environment. For instance, sanitary processes often require isolation, and sorting animals into new groups generally entails breaking relationships with familiar conspecifics as well as encountering unfamiliar ones. Encountering unfamiliar animals or simple isolation are causes of marked stress reactions in the majority of domestic species that are known to be gregarious (Boissy et al., 2001). Additionally, social isolation is one of the most stressful components of many fear tests when members of a social species are tested individually. Most domestic species are strongly motivated to rejoin conspecifics when isolated and may consequently suffer more from separation anxiety than from the presumed fear-eliciting event itself.

Diversity in fear-related responses

Fear-related behaviors vary greatly depending on the nature of the threat. They can sometimes seem contradictory, as both active and passive strategies may be observed in a challenging situation: these strategies include active defense (attack, threat), active avoidance (flight, hiding, escape), or passive avoidance (immobility) (Erhard and Mendl, 1999). Subtle expressions can also be regarded as fear indicators; for example, expressive movements such as head postures and facial expressions, alarm calls, and the release of alarm odors or pheromones. These fear-related responses play important social roles by serving as alerting signals for conspecifics. Few studies that have been recently carried out in ruminants provide some insight that ear postures

may be useful in assessing emotions in farm animals. In cattle, a high occurrence of pendulous ear postures was used as an indicator of the animals' positive rating of their favorite grooming sites (Schmied et al., 2008). We found that sheep point their ears backward when they face an unfamiliar or an unpleasant event and they react to a sudden event by an asymmetric posture of their ears (Boissy et al., 2011). These first results offer a promising step in the characterization of specific emotion-related facial expressions in farm animals as measuring ear postures constitutes a reliable noninvasive method for monitoring the emotional reactions in animals.

Fear-eliciting events can also affect the activity in which the animal is engaged: low levels of fear can enhance activity (e.g., attention, investigation) whereas intense fear can disturb or terminate an ongoing activity. Indeed, fear inhibits all other behavior systems including feeding, exploration, sexual, and social interactions (Jones and Boissy, 2010). Finally, the conflict between a negative emotional state and a positive motivation may generate a compulsive behavior; for example, a disturbed or hungry pig bites a chain or bar. Activation of the sympathetic nervous system, including the adrenal medulla, and the hypothalamic−pituitary−adrenal system are major neuroendocrine responses associated with negative emotions (Mormède et al., 2007; von Borell et al., 2007) but they also accompany positive rewards such as food delivery, sexual interaction. Frightening stimulation also elicits a range of complex changes in central nervous mechanisms, such as neural pathways and neurotransmitters (Gray, 1987; Phillips et al., 2003; Rosen, 2004).

Various ways of assessing fear and anxiety

Varied experimental situations have been designed to study fear in farm animals and most of them were originally developed for laboratory species (for a review, see Forkman et al., 2007). Since Hall's classic work (1934), the open-field or novel arena test has been extensively used in rodents. Generally, a single animal is placed in a large novel arena and the amount of defecation and activity is interpreted as reflecting the emotional response to novelty. Later work showed that this test also incorporates many other threatening stimuli, such as absence of shelter and landmarks, human contact, social isolation, and bright light. Many other paradigms devised to assess fear in rodents, for example, exposure to a predator or a novel object, confinement, handling, inescapable noxious stimulation, and passive or active avoidance conditioning (Ramos et al., 1997) have also been used in farm animals from the 1970s onwards (Forkman et al., 2007) though the designs were generally modified to suit the circumstances. Fear of novelty is evaluated using the open-field test or exposure to a novel object while few specific tests have been developed to assess an animal's fear of humans, such as the forced approach test or the voluntary approach test. The forced approach

test is more likely to elicit an active response whereas the likelihood of observing either no response or a passive one is greater in the voluntary approach test (Waiblinger et al., 2006). Restraint tests are also commonly used, for example, by restraining the animal in a chute or, more commonly, by inducing a tonic immobility reaction (Forkman et al., 2007). Finally, fear can be induced and evaluated in terms of the animals' reactions when exposed either to a natural predator, or to a sudden event (i.e., startle test), or to a signal that had been previously associated with a nociceptive event such as electric shock (i.e., conditioned fear test).

A need for a refined and structured methodology in fear studies

Many tests of fear were originally designed for laboratory rodents and, unfortunately, several of these have been used for farm animals with insufficient regard for their biological relevance. For example, while laboratory rodents are nocturnal and wall-seeking (wild rats build and live in burrows) most farm animals are diurnal and, apart from poultry, their ancestors generally occupied open areas/ranges. Cattle may actually perceive the open-field test as an enclosed area. Furthermore, most farm animals are highly gregarious and react heavily to being separated from group-mates, many have exclusive mother−young relationships and the young are generally precocious. In view of such species differences in ecological experience and motivation, we must avoid testing animals in inappropriate environments that may elicit motivational states unrelated to the one under study, thereby leading to inaccurate estimations of fear and inconsistencies between studies. Reconsideration of the ecological contexts of domestic species may help us develop more reliable and valid tests and measures of fear.

Despite these cautionary notes, studies of laboratory animals may still guide the interpretation of a range of emotional reactions observed when farm animals are exposed to aversive situations. From an ecological perspective, suddenness and novelty, which underpin many fear tests (Boissy, 1998), are key features of predatory attack and we must remember that domestic ungulates and poultry kept free-range may still experience predation by wild animals and dogs (Shelton and Wade, 1979). The ability to cope with an aversive event can also strongly influence the animal's emotional experience. For instance, cows with the strongest tendency to approach a novel object voluntarily are also the most reactive to humans, but the opposite is true if they are forced to move toward that novel object (Murphey et al., 1981).

The expressions of fear, like those of all the emotions, are brief reactions that are often difficult to measure, particularly in farm animals kept in commercial conditions. According to Forkman et al. (2007), research in this area would benefit substantially if more effort was devoted to overcoming three particular weaknesses. First, many tests used to measure fear need more rigorous examination of validity and repeatability. Validation through

evaluation of intertest consistency should thus be given more attention in future studies (Forkman et al., 2007). Second, there is a pressing need for standardized protocols to ensure the harmonization of methods across research teams and countries. This would reduce unnecessary duplication of effort while greatly facilitating the meaningful interpretation of the results and their general acceptance. Third, the protocols have to be robust and practical: it must be possible to carry them out in an acceptable time frame, at the farm level, and without causing too much disturbance to the farmers' work. Therefore it is not enough simply to transfer a laboratory test to the field without making appropriate modifications to fit the animals' biology and needs, the environmental conditions, and the farmers' requirements.

A recent comprehensive review of the methodologies used to assess fear (Forkman et al., 2007) in farm animals was a very useful first step in bringing together some of the above-mentioned issues; it describes the various methods used for cattle, pigs, horses, sheep, goats, chickens, and quail and gives indications of their repeatability and validity. Similarly, fear tests involving exposure to humans were discussed in a review of human−animal interactions (Waiblinger et al., 2006). The Welfare Quality project (2009), a multidisciplinary EU-funded study, also made substantial progress in developing, refining, validating, and standardizing numerous animal-based measures of welfare, including fear-related behaviors. However, there is still a need for continued development and refinement of fear-related measures.

Contribution of cognitive psychology to access animal emotions

Emotion is any mental experience with high intensity and high hedonic content (i.e., the negative or positive valence). In humans, the emotional experience is generally inferred from verbal self-reports. As conceptual-psychological scales cannot be used in animals that have no verbal language, their emotional experience can thus only be inferred from their behavioral and physiological reactions. Emotions have both arousal and valence dimensions. However, behavioral and physiological reactions generally provide, as it has been reported in the previous section, a quantitative assessment of the emotional activation (i.e., the arousal of the emotional response), but they do not allow defining the exact nature of the emotion (i.e., the negative or positive valence) felt by the animal. For example, plasmatic concentrations of glucocorticoid can be increased both in response to acute negative emotional stimuli (Mason, 1971) or to the expectation of a positive situation such as the sexual partner (Colborn et al., 1991). Likewise, common behavioral reactions such as startle, offensive or defensive postures, freezing, or approach, only provide information about the intensity of the underlying emotion (for a review, see Jones and Boissy, 2010). Therefore the study of emotions in animals is mostly addressed by means of defining the response of a subject to an emotionally arousing situation. In this way, the emotional reaction is

measured by behavioral and physiological parameters without requiring any preconceived theory about what emotions really are. Conceptual frameworks are thus needed to allow a convenient and reliable assessment of emotion in animals.

Among possible conceptual frameworks, appraisal theories originally developed in cognitive psychology to investigate human emotions can be helpful to better access the emotional experience in animals (Boissy et al., 2007a; DéSiré et al., 2002). Furthermore, there is increasing evidence that causal links between emotions and cognition occur in both directions, with not only cognitive processes determining felt emotions but also with emotional manipulations more or less persistently influencing cognitive processes (Mendl et al., 2009; Paul et al., 2005). Below we provide a brief review of the interactions between emotions and cognition in farm animals. Firstly, we examine the cognitive processes involved in the development of emotions. Then, we explore how emotions may in turn alter these cognitive processes and how short-lived processes such as emotions can lead to prolonged affective states.

Influence of cognitive processes on emotions

Since the pioneering work of Magda Arnold, appraisal theories have been developed in psychology to access emotions in humans: emotions result from how an individual evaluates a triggering situation per se followed by his responses to that situation (for a review, see Kappas, 2006; Lazarus, 1991; Scherer, 1999). According to the pragmatic framework developed by Scherer (2001), this evaluation is based upon a limited number of elementary characteristics, from the most simple to the more complex, including (1) the characteristics of the event (its suddenness, familiarity, predictability, and pleasantness), (2) the consistency of the event with the individual's expectations, (3) the coping potential, mainly the ability of the individual to react and to control the event, and (4) the normative significance of his response, including his internal standards (i.e., his self-esteem) and his social standards (i.e., the expectation of the social group). The outcomes of these elementary characteristics determine the emotional experience. For instance, fear is elicited by exposure to an unpleasant event that is sudden, unfamiliar, unpredictable, and inconsistent with the expectations of the individual, whereas rage is experienced in similar situations, except that the individual's evaluation is that he can control the challenging situation (Sander et al., 2005).

The framework based on appraisal theories was recently transposed to animals (DéSiré et al., 2002). We developed various experimental situations in sheep that were designed to activate one by one the evaluative characteristics, to ascertain which ones are relevant to animals (Boissy et al., 2007a; Veissier et al., 2009). Cardiac and behavioral reactions were recorded to probe the links between presumed appraisal and measurable emotional

outcomes. As already reported in many species, it was found that sheep reacted to sudden and novel events (DéSiré et al., 2004, 2006). The evaluation of these elementary characteristics appears rather automatic and may not require the animal to be aware of its evaluation of the situation. More interestingly, it was found that the emotional responses of sheep (e.g., behavioral and cardiac responses) are also affected by the predictability of a situation, its controllability, or its consistency with the animal's expectations (Greiveldinger et al., 2007, 2009, 2011). More recently, we found that the emotional responses to a threatening event are influenced by the animal's social context (Greiveldinger et al., 2012). Exposed to a sudden event (a panel falling down behind a trough when the animal is eating), sheep are more likely to display overt external responses (stepping back from the trough) when they are accompanied by a subordinate group-mate, but internal responses (tachycardia) when they are accompanied by a dominant group-mate. Therefore it is clear that sheep are responsive to the main appraisal characteristics defined in human studies. Although this approach has not been completed for many farm animals, it is now widely accepted that not only mammals but also poultry can feel emotions (Mormède et al., 2007; Valance et al., 2008).

As animals can use appraisal processes similar to humans, we can consider that they not only express emotional responses that in that case could be considered reflexes, but they really do feel emotions (Veissier et al., 2009). This is in line with several studies that highlight the existence of mutual neural circuits underlying experience and expression of emotions in both humans and animals (Boissy et al., 2007a; Spruijt, 2001). Referring to the studies conducted in sheep and based on the elementary characteristics sheep use to evaluate their environment, it is suggested that they can experience a wide range of emotions: (1) fear and anger, as they are sensitive to suddenness, unpredictability, controllability, and social norms; (2) rage, as they respond to suddenness, unfamiliarity, unpredictability, discrepancy from expectations, controllability, and social norms; (3) despair, as they react to suddenness, unfamiliarity, unpredictability, discrepancy from expectations, and controllability; and (4) boredom, as they are sensitive to suddenness, unfamiliarity, unpredictability, discrepancy from expectations, and controllability (Veissier et al., 2009). Using a similar pragmatic framework in various farm animal species, comparisons across phyla could provide critical insights into the kind of emotions they can feel and thereby help to refine regulations designed to safeguard animal welfare.

Alteration of the judgment and decision-making by emotions

While cognitive processes are at the origin of emotions, emotions can in turn influence cognitive processes. Here again, a significant body of work in human psychology has shown how emotions can temporarily bias the

processing of information coming from the situation that is attention, memory, and judgment. For example, anxiety induces a shift in attention towards potential threats (Bradley et al., 1997), emotionally charged events are more readily remembered than neutral ones (Reisberg and Heuer, 1995), and people exposed to strongly negative events tend to judge all subsequent ambiguous events negatively (Wright and Bower, 1992). Such cognitive biases induced by an emotion may have adaptive value by helping individuals to pay attention and to memorize threatening circumstances. They are not restricted to humans (Paul et al., 2005). In rodents, the startle response induced by exposure to a sudden event is faster and larger under negative emotional states (Lang et al., 1998). Heifers subjected to a potent stressor were unable to abandon a previously learned behavior that was no longer rewarded; this prevented them from acquiring a new, more appropriate behavior (Lensink et al., 2006). Sheep experiencing repeated aversive events demonstrated learning deficits (Destrez et al., 2013). In contrast, rats given a catecholamine injection mimicking the physiological component of a moderate emotion are more attentive and display improved memory (Sandi et al., 1997).

The evidence that an emotion has an immediate and temporary repercussion for cognitive functions suggests that it would be worthwhile to study how an accumulation of emotions modifies an animal's cognitive functions in a long-lasting manner. Rats or mice previously subjected to repeated frightening events respond less readily to an ambiguous stimulus signaling the delivery of a positive event long after the last exposure to a frightening event, thus revealing a persistent reduction of the capacities for judgment and decision-making (Harding et al., 2004; Pardon et al., 2000). Similarly, rats housed under social stress show less behavioral agitation after the presentation of a conditioned stimulus predicting the delivery of sucrose reward, suggesting reduced anticipation due to impaired judgment (von Frijtag et al., 2000). Likewise, sheep submitted to repeated unpredictable aversive events for several weeks are less prone to respond to a stimulus signaling the delivery of a positive or negative event, especially when the signal is ambiguous, that is, between a positive and a negative signal (Doyle et al., 2011; Harding et al., 2004). These findings suggest that the accumulation of negative experiences affects the way the animal appraises the environment to which it is exposed, making it less aware of positive cues and more susceptible to negative ones. Such persistent biases (optimistic vs pessimistic) guide the animal's decisions when appraising new situations or events, especially if there is a degree of ambiguity in their potentially rewarding or punishing consequences (Mendl et al., 2009). If an animal lives in an environment where it has experienced fear several times, it would make adaptive sense to appraise ambiguous events as more likely to be negative and hence to take safety-first avoidance action. Such persistent biases that could be used as a noninvasive indicator of comfort or distress merit further investigations.

Establishing the nature and frequency of cognitive processes used to evaluate the environment may help us to understand why chronic stress sometimes results in apathy or blunted emotion while in other cases it leads to heightened emotional reactivity (Boissy et al., 2001). Apathy would likely develop when the animal has no way of altering negative events, whereas hyperreactivity would occur when it thinks it can control such events.

In conclusion, the study of complex interactions between emotions and cognitive functions offers a new impulse to the study of affective states in animals. Few integrative and multidisciplinary (behavior, psychology, physiology, and neurobiology) approaches exist now to objectively assess the affective states of animals in terms of welfare. In addition to studying the role of cognition in the generation of emotions, studies on the effects of emotions on cognitive biases should be promoted to provide deeper insight into the relationships between emotions and more persistent affective states close to welfare. Taken together, all these studies demonstrate how cognitive approaches can be used in animals to probe emotions as short-term affective experiences and welfare as persistent affective experiences.

Animal individuality in emotions: the concept of personality

Not all individuals will react in the same way to challenging situations. They will be more or less afraid, more or less curious, more or less difficult to handle, and will react more or less strongly to frightening situations. For instance, there were marked individual differences in the reactions of beef cattle to frightening situations (Kilgour et al., 2006). Nobody who has ever worked with animals will question the fact that there is a considerable variation in how individuals behave in a given situation, that is, in the "state" they are in, and that this variation is, to some extent, stable across time and situations. When we find such stability, we can call it a "trait," such as fearfulness, docility, and aggressiveness. We can then look for ways of assessing them and for underlying causes for the variation, such as genetic or developmental effects.

In the last 20 years or so, animal scientists have tried to catch up with what stockpeople have known since domestication began. And yet, they have not managed to agree on a label for this variation (Gosling, 2001). In this chapter, we will use the term "personality," as it has been shown repeatedly that consistent individual differences in behavior of human and non-human animals are sufficiently similar to be described by the same label (Gosling and John, 1999; Gosling, 2001; Mehta and Gosling, 2006). There are many definitions of "personality"; the one proposed by Pervin and John (1997; cited by Gosling, 2001), "consistent patterns of feeling, thinking, and behaving," incorporates three different elements, namely emotion, cognition, and behavior. Likewise, whereas dimensions of personality are generally discussed in terms of coping style or fearfulness, it has been proposed that there

is currently no single concrete method for assessing a propensity to feel and express emotions (Gosling, 2001).

Individual variability in emotional behaviors

To qualify for the label "personality," individual differences have to be consistent over time and across situations (Erhard and Schouten, 2001), which are typically social isolation, novelty, suddenness, and predator response (in many cases, humans may evoke the same reactions as predators). Comprehensive reviews of the literature on fear tests used on farm animals (Boissy, 1998; Dodd et al., 2012; Forkman et al., 2007; Jones and Boissy, 2010) already exist and we will just present some newer findings in the field in this section. Individual differences in fearfulness are not just interesting from a theoretical or animal welfare point of view. High levels of fear can make animals difficult and dangerous to handle.

Consistency of fear-related responses across different frightening situations

In animal experimentation, this element of personality is referred to as the "validity" of a behavior test (Forkman et al., 2007). It is based on the notion that a personality trait will be expressed in a similar way in different situations within the same context. For example, sheep that were prepared to move away from their group to explore an unknown environment in a behavior test were found to have a lower group cohesion 9 weeks later when grazing undisturbed. They were less easily frightened, recovered more easily from a fright (Sibbald et al., 2009), and split into subgroups more easily (Michelena et al., 2009) than sheep that explored less. While not all behavior variables may be correlated across tests, there is often sufficient evidence to suggest a common trait, in that individuals that react more strongly in one situation will also react more strongly in others. In horses, for instance, the frequency of licking/nibbling a novel object, the time to put one foot on a novel area and to eat from a bucket placed just behind it, and the flight distance and the time to eat under an opened umbrella were found to be interrelated and consistent over time (Lansade et al., 2008a).

Not all reports show a strong consistency, however. This can have several reasons: maybe the test is not appropriate, or the situations belong to different contexts. Another possibility is that the same underlying fear may result in different behaviors, depending on the situation. When horses were confronted with a range of frightening stimuli, such as a traffic cone, white noise, and the smell of eucalyptus oil, the resulting heart rate was correlated between situations while the behaviors were not. The authors explained this by a nondifferentiated activation of the sympathetic nervous system (heart rate), and a stimulus-specific link of the behavior (Christensen et al., 2005).

Many situations give rise to several aspects of personality. Calves placed into an open field (social isolation in a novel environment) and confronted with novel objects showed avoidance and cortisol responses that were interrelated. These were interpreted as reflecting underlying fearfulness. Locomotion and vocalization in the open-field, however, were independent and interpreted as reflecting other personality traits, such as activity and sociality (Van Reenen et al., 2005). Fear of people may also increase a female's motivation to defend its offspring, with dangerous consequences for the stockperson (Turner and Lawrence, 2007).

Consistency of fear-related responses over time

The concept of personality includes an element of consistency over time. In horses that were put in social isolation, the frequency of neighing was found to be well correlated with other behaviors displayed in the same test, but also over time, from 8 months to 2.5 years old (Lansade et al., 2008b). A similar result was found for the horses' reaction to humans. Various behavioral reactions—such as the frequency of licking/nibbling a person, the time taken to touch the horse and to fit a halter—were significantly correlated across time (Lansade and Bouissou, 2008).

Beef cattle going through a handling system (squeeze chute) they were familiar with showed consistent differences in flight speed when tested twice on the same day, but also 1 month apart (Müller and Von Keyserlingk, 2006). In another study, the flight speed showed consistency over time, the behavior while in the crush did not (Cafe et al., 2011). In another study (Benhajali et al., 2010), however, the behavior during constraint in a chute was repeatable across years. The cattle used were of very different genetic backgrounds and from different production systems (Canada, Australia, and France). It is, therefore, not always possible to generalize tests from one system/breed to another. While we are recording the behavior of the animals, we must not forget that this behavior is a result of underlying emotions, which are dependent on how the animals interpret the situation (see the previous section). The reaction of a dairy cow and of a beef cow from an extensive range system towards a human will not be the same. While in one case the human is familiar and may be associated with positive experiences, in the other he might be regarded as a predator.

Personality as a complex intermediate variable

As suggested throughout this chapter, behavioral reactions and physiological patterns observed in animals under emotional challenges are only indicators of fear/anxiety and cannot be considered as direct measures of a fear/anxiety state. Fear must be evaluated by studying not only the intensity of a unique response but also the overall strategy. The different mechanisms underlying

fear-related responses probably depend on other motivational systems that may modulate these responses. The strength of a reaction in response to a stressor is often used to assess the strength of the underlying emotion. The classic example is the fight/flight response that assumes that a higher level of activity indicates a higher level of fear. However, an absence of activity, immobility, also indicates fear. This might explain the negative correlation between activity while confined and in social isolation and the fear of humans in sheep (Beausoleil et al., 2008). The interaction of several personality traits, such as fearfulness, activity, and coping strategies (active/passive) can explain these complex results (Erhard et al., 1999; Koolhaas et al., 1999; van Reenen et al., 2005).

Vocalizations are often considered to be an indicator of fear: the more an animal vocalizes, the more stressed it probably is. Vocalizations, however, are also a means of communication and could be an indicator of sociability. Because of the complexity of the phenomena that can affect emotional behavior, it is not possible to just consider the results isolated from their context. A measurement used as a fear indicator in one situation cannot be directly extrapolated to others, and it is impossible to simply assess the magnitude of concepts like fear and anxiety on the basis of a single "objective and perfect measurement."

Pre-existing characteristics of temperament

Genetic background and genetic models of emotional reactivity

Although farm animals have been domesticated over many centuries, fear-related behaviors still vary considerably both within and between populations (Boissy et al., 2005b; Hemsworth, 2003). An animal's genetic background plays a major role in determining its fear levels and its ability to adapt to environmental changes and challenges. Large differences in emotional reactivity are also apparent within and between farm animal breeds (Boissy and Bouissou, 1995; Boissy et al., 2005a; Faure et al., 2003). The approaches go from comparison of breeds (Hansen et al., 2001) to estimation of genetic correlations and heritability within the same breed (Benhajali et al., 2010), selection lines (mink: Malmkvist and Hansen, 2002; sheep: Beausoleil et al., 2008, 2012), and the study of quantitative trait loci (QTLs) (Gutiérrez-Gil et al., 2008; Hazard et al., 2012; Jensen et al., 2008). Estimates of the heritability of fear in domestic animals seem sufficiently high to allow further selection against fearfulness (Boissy et al., 2005a). Observed heritabilities also revealed a genetic component in calf reactivity (behavior, heart rate, cortisol) to a fear test; fearful calves became fearful heifers, and high fearfulness and a poor milking temperament were associated with low milk yield (Jones and Manteca, 2009; Van Reenen et al., 2005; Van Reenen et al., 2009). For a further discussion of the genetic aspect of fearfulness, see the section on *Genetics and Selective Breeding for Alleviating Negative Emotions*.

Developmental aspects

While there is a strong genetic component to fearfulness, the environment plays a very important role as well. The role of the prenatal environment for the development of farm animals has recently been reviewed (Roussel et al., 2007; Rutherford et al., 2012). The role of the hypothalamus−pituitary−adrenal axis is of particular interest (Kapoor et al., 2006; Matthews, 2002). The main aspects are maternal stress and nutritional state during pregnancy, and the effects depend on the severity of the stressor as well as on the developmental stage of the fetus and the time of the stressor. Periconceptional undernutrition in sheep (Hernandez et al., 2010), as well as undernutrition in early (Erhard et al., 2004) and late gestation (Laporte-Broux et al., 2012), can affect the development of fearfulness in the offspring to affect them throughout their lives. Mammals are not the only ones affected by prenatal stress. Maternal stress in birds (i.e. preegg laying) results in more fearful offspring (Henriksen et al., 2011; Janczak et al., 2007). Even in farmed fish (salmon), there is evidence for the deleterious effects of maternal stress on the development of the offspring (Eriksen et al., 2011; Espmark et al., 2008).

The development of fearfulness does not end at birth. More fearful quail, for instance, can still increase their chicks' fearfulness posthatching (Richard-Yris et al., 2005). There are periods when young animals are specifically sensitive to long-term changes in fearfulness, for instance around weaning (Lansade et al., 2004).

Personality and health

There is evidence of a link between personality and health in animals (Koolhaas, 2008; Mehta and Gosling, 2008). Individuals scoring high on emotional reactivity and fearfulness are more easily stressed and, as a consequence, may have a weaker immune system. For instance, fear-related reactions to humans in cattle entering a feedlot are negatively associated with immune function (Fel et al., 2003). The reality can, however, be more complicated. Pigs that react more actively to restraint-stress at a young age show a stronger immune response several weeks later, compared to counterparts that are less active to the restraint test. The latter, however, seem to be more strongly affected by their housing environment at the time of the immune challenge (Bolhuis et al., 2003).

In conclusion, there is overwhelming evidence that stable individual characteristics exist in animals, in the strength of emotions they show and in how they react to environmental challenges. These characteristics are influenced by genetics, but also by developmental factors, pre- and postnatally. To get animals that are easy and safe to work with and that have good welfare, the genetic background, but also the environment of the parent generation, must not be neglected.

A concept of positive welfare based on positive experiences

Should we adapt the environment to suit the animal or change the animal to suit the environment? Both approaches can be helpful. On the one hand, we know that appropriate manipulation of environmental factors can profoundly change an animal's behavioral and physiological capabilities. Indeed, good stockmanship and environmental enrichment can improve the animals' quality of life (Boissy et al., 2007b; Duncan, 1996; Fraser and Duncan, 1998; Hemsworth and Coleman, 1998; Jones, 2004). On the other hand, given the substantial genetic diversity within and between populations, selective breeding probably represents the quickest, most reliable method of reducing fear and other welfare problems. The examples of heritabilities for fearfulness reported in the previous section suggest sensitivity to genetic manipulation in farm animals (Boissy et al., 2005b; Faure et al., 2003; Rodenburg and Turner, 2012). In reality, an integrated approach involving targeted breeding programs, environmental manipulation, and improved management is likely to be the most effective strategy (Boissy et al., 2005b; Faure and Jones, 2004; Jones, 2004). For instance, while depression-like behaviors are expected after chronic stress, a significant proportion of animals (up to a third or half of samples) are resistant and do not develop these symptoms. Therefore the emotional state of an animal seems to result from both its environment (especially previous stressful experiences) and its own individual characteristics.

We briefly report the genetic approaches for alleviating negative emotions, and then we present the common strategies of enrichment to induce positive emotions in livestock animals before introducing promising behavioral strategies based on evaluative abilities of the animals to improve their welfare and possibly their health.

Genetics and selective breeding for alleviating negative emotions

Farm animal welfare management generally focused on minimizing disturbances that generate unpleasant emotions and longer-lasting affective states. Breeding and genetics have played an important role in such an approach. Negative emotions and defensive behaviors might be reduced or eliminated by using genetic stocks that either does not show these behaviors or exhibit them so little or so infrequently as to be of trivial importance (Craig and Swanson, 1994). Genetic selection could be a powerful tool for decreasing the incidence of behaviors associated with welfare problems, such as fear. Indeed, selective breeding programs for reduced fear or dampened stress responsiveness have already been established in turkeys (Brown, 1974) and Japanese quail (Jones and Satterlee, 1996; Mills and Faure, 1991). For example, divergent selection of Japanese quail either for short tonic

immobility fear reactions (Mills and Faure, 1991) or a decreased plasma corticosterone response to restraint (Jones and Satterlee, 1996) led to reductions in their fear and physiological stress responses to a wide range of potentially traumatic situations (Jones and Satterlee, 1996; Mills and Faure, 1991). Likewise, marker-assisted selection may be a useful tool for manipulating emotional states (Boissy et al., 2005b). For example, QTLs for fear (Schmutz et al., 2001), for reactivity to humans (Davis and Denise, 1998), and for social reactivity (Hazard et al., 2012) were identified in cattle and sheep using linked markers. Such linked markers could, in turn, be used for marker-assisted selection within sire families once the relationship between the marker and the gene has been determined. Genomic tools could thus facilitate selection for complex behavioral traits, which are frequently impossible to measure on a large number of animals. Identifying and manipulating fundamental behavioral traits that underlie adaptation to the physical and social farming environment might be an effective strategy for improving farm animal welfare in a broad sense as well as safeguarding or enhancing production, product quality, and profitability.

Some concerns must be addressed before genetic selection is widely accepted as a welfare-friendly tool. Firstly, as selection for one trait may also modify other characteristics, it is essential to ensure that there are no associated undesirable effects on welfare; new breeding tools can indeed enhance the rate of undesirable change in correlated traits of welfare significance (Rodenburg and Turner, 2012). Secondly, it is necessary to take into account the ethical objections that are raised regarding genetic selection. However; it is uncertain whether these objections make the distinction between selective breeding (practiced since animals were first domesticated) and genetic manipulation (e.g., gene insertion or deletion). Of course, interactions between the animals' genotype and their environment are critical determinants of animal welfare that may help reconcile production with ethical concerns (Boissy et al., 2005b). Briefly, we regard genetic selection for reduced fearfulness as ethically sound because it could increase the animal's ability to interact successfully with its physical and social environment by dampening the inhibitory effects of fear and alleviating distress. This not only reduces the animals' stress but also facilitates their management (better human−animal relationship) and consequently increases their welfare.

Eliciting positive emotions by enriching the environment and management

Traditionally, the focus has been on the negative side of animal welfare that is acute fear and chronic stress (see earlier). This strategy produced animal welfare benefits, but at best it could only lift a poor welfare status to a neutral one. However, welfare is based on a balance between negative and positive experiences (Dawkins, 1990; Duncan, 1996; Mellor, 2012; Spruijt et al., 2001),

and it is now widely accepted that positive welfare is not simply the absence or reduction of negative experiences but needs also the expression of positive ones such as pleasure and comfort (for a review, see Boissy et al., 2007b). Therefore in addition to the assessment of negative experiences, it is now necessary to consider both positive expectations (what an animal "likes") and resources that an animal is motivated to obtain (what an animal "wants"). However, despite the efforts of pioneering authors such as Colin Allen, Jaak Panksepp, or Kent Berridge, relatively little had been done until recently to understand positive experiences in comparison with their negative counterparts. As an emerging field in animal welfare, there is now a growing interest in positive emotions and enrichment in captive animals. We present the current efforts to induce positive animal welfare based on environmental enrichment and the improvement of human–animal relationships.

Environmental enrichment

Although it is often recommended that the animals' environment should be predictable and controllable (Bassett and Buchanan-Smith, 2007), an invariable and overly predictable one should be avoided as it may result in boredom (Van Rooijen, 1984). Environmental enrichment generally involves incorporating new, putatively interesting stimuli in the home environment and thereby increasing its complexity and stimulus value. This promotes desirable behaviors such as foraging, exploration, social play, and grooming in pigs (Bracke et al., 2006; Spinka et al., 2001; Van de Weerd et al., 2003), cattle (Wilson et al., 2002), and poultry (Jones, 2004), reduces excitability and fear in pigs (Grandin et al., 1987) and sheep (Vandenheede and Bouissou, 1998), as well as reduces neophobia in poultry (Jones, 2004). There is mounting evidence that animals housed in enriched conditions have more positive affective states compared to those housed in more barren environments. This belief is supported by the cognitive bias assessments that report more optimistic cognitive biases in laboratory rats currently housed in more enriched or more stable cages (Brydges et al., 2011; Harding et al., 2004). Similar results have been reported in farm animals. For instance, pigs that have spent time in an enriched environment have more optimistic judgment biases, indicative of a more positive affective state, in comparison with counterparts reared in a barren environment (Douglas et al., 2012).

However, there are several critical requirements, particularly in the farming sector. Practicality is paramount, for example, enrichment devices must be safe (no sharp edges or protrusions), hygienic, affordable, durable, accessible to all members of the group, and retain interest as long as possible to reduce the need for frequent change. Ideally, we should determine what sorts of stimuli the animals find attractive and interesting (Bracke et al., 2006; Jones, 2004; Wilson et al., 2002). Important questions include: Are there unlearned preferences? Are movement and change important? Is physical

interaction with the device essential? At what rates of introduction and stages of the animals' development would enrichment be most beneficial? Identifying the factors governing an animal's perception of a safe and interesting world would greatly facilitate the development of effective environmental enrichment.

Positive human contact and training programs

Poor human−animal relationships cause the animals to be fearful of humans (Manteca et al., 2009). In some production systems (e.g., extensive farming), the animals are less likely to experience human contact that could in turn make them even more fearful of people through a lack of habituation (Boissy et al., 2005b). Remedial efforts for more tractable animals focus on two main approaches, which can also be integrated in a complementary fashion: (1) regular and positive human contact and (2) stockmanship training programs.

Picking an animal up regularly, stroking it, or giving it food reliably reduces fear of people in farm mammals (Coulon et al., 2012; Hemsworth et al., 1993). Nevertheless, it is clearly not a practical option in modern agriculture with the tendency to increase the herd size and by contrast to reduce the labor force. Encouragingly though, simply allowing regular visual contact with people reduced fear of humans in lambs (Tallet et al., 2008). Training programs are the main strategy for achieving and maintaining a good human−animal relationship (Hemsworth, 2003). A team of European and Australian researchers in the EU-funded Welfare Quality project (http://www.welfarequality.net) developed a multimedia-based training package (courses, software, manuals, newsletters, etc.) designed to help stockpersons improve the quality of their interactions with cattle, pigs, and chickens, and hence enhance the human−animal relationship (Boivin and Ruis, 2011).

Cognitive enrichment: a new approach based on the animals' appraisal abilities

The conceptual framework that has been developed from recent advances in cognitive psychology and that is reported earlier (see the section, *Contribution of Cognitive Psychology to Access Animal Emotions*) is particularly useful to scientifically explore positive feelings in animals and to develop strategies in animal husbandry for enhancing positive emotional experiences. We outline three specific cognitive processes for eliciting positive experiences: signaling a reward in advance, giving a higher reward than expected, and enabling the animals to cope with or to control a wanted event (Boissy et al., 2007b).

Investigations of what animals find positively reinforcing and of the animal's behavioral expression during anticipation or expectation of the

rewarding result, often called positive anticipation, provide a basis for assessing positive emotional states in animals (Spruijt et al., 2001). When anticipating food rewards, rats (van der Harst et al., 2003), mink (Vinke et al., 2004), poultry (Moe et al., 2009), and pigs (Dudink et al., 2005) show increased locomotor activity and frequent behavioral transitions, that is, anticipatory hyperactivity, which reflects positive emotional experience. In pigs, signaled positive stimuli such as extra space, food, or straw lead to increased activity due to an orientation toward the place where the reward is offered and frequent behavioral transitions, most often combined with play markers, that is, hopping, scampering, pivoting, pawing, flopping, and head tossing (Dudink et al., 2005).

In a typical positive contrast experiment, an animal is first trained to perform a task by giving it a reinforcer of a certain size (for a review, see Flaherty, 1996). The animal is then given a larger reinforcer than it had been given before. If the animal changes its behavior so that its response is faster/more vigorous than that of a control group given the larger reinforcer from the start, a successive positive contrast has occurred. Positive contrast can be found for both appetitive and consummatory responses. It is the difference between the expected value and the actual value that influences a subject's behavior rather than the exact level of reinforcement. In his original study, Crespi (1942) reported that rats for which the quantity of reward has just been increased run faster towards the food reward than do rats having always received the large quantity of reward. Similarly, sheep showed transient hyperactivity if a reward was of greater hedonic value than expected (Greiveldinger et al., 2011). Nevertheless, due to the relatively small number of studies, further research is needed to confirm the value of a positive contrast model in the study of potential positive emotions and their behavioral expressions.

Enabling the animals to cope with or to control a wanted event (controllability of reward) may be another source of positive emotions. Social and physical challenges can be perceived as positive experiences provided there is an adequate coping behavior. Meehan and Mench (2007) suggested that successful dealing with adequate cognitive challenges can induce positive emotion. Hence, frequent challenges that can always be successfully mastered and eventually enable the animal to reach a desired and rewarding goal may be suitable means to regularly elicit positive emotions. Using such a cognitive approach, a program of research on the effects of rewarded cognitive processes has been developed (Ernst et al., 2005): each animal out of a group of pigs had to learn an individual acoustic signal as a call to work for food by pressing a button. Using this approach, Zebunke et al. (2011) report transient cardiac reactions revealing positive emotional experiences in pigs. Interestingly, pigs reared for several weeks under such a complex but predictable environment presenting positive challenges with which individuals are able to cope, present modifications in the reward-sensitive brain

opioid receptors compared to conventionally housed pigs, indicating frequently occurring positive experiences (Kalbe and Puppe, 2010). In conclusion, this brief review on cognitive environmental enrichment provides valuable insights into the eliciting of positive emotions in animals. Based on the principle of animal-based measurements, adequate cognitive challenges are evaluated as emotionally positive by the tested animals.

Promoting positive experiences to mitigate negative experiences and to improve animal welfare and health

Cognitive enrichment, such as anticipatory behavior, might be useful in developing a behavioral therapy to counteract the deleterious effects of stress. For instance, rats submitted to prolonged stress did not develop anhedonia—a major symptom of depression—if they received repeated food rewards announcements, suggesting that positive experiences can counteract the deleterious consequences of negative experiences (van der Harst et al., 2005). Thus signals of reward might form the basis for a new enrichment strategy. Likewise, reduction of fearfulness reduces pessimistic-like judgment in lambs compared with stressed counterparts (Destrez et al., 2012). Furthermore, induction of anticipation via the announcement of certain alarming events might also be a way to reduce the negative emotion induced by such events. For instance, sheep for which the appearance of a sudden event was preceded by a light signal expressed fewer fear reactions than their counterparts that could not anticipate (Greiveldinger et al., 2007). Therefore the implementation of cognitive challenges into animal housing, based on positive anticipation, positive contrast, and positive control, is a promising approach to induce long-lasting positive effects on animal welfare.

Although welfare and health are distinct concepts, they influence each other. Considering health as a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity,[1] it is essential to reconsider the management of animal health through an integrated view, particularly by taking into consideration animal emotions and welfare/stress. There are permanent interactions between the immune system and the brain, notably through the inflammatory cytokines and the autonomic nervous and neuroendocrine systems. Although research in this area is recent, there is evidence that chronic stress results in glucocorticoid receptor resistance that induces the exaggerated local release of inflammatory cytokines, resulting in increased disease risk because inflammation plays an important role in the onset and progression of a wide range of diseases (for a review, see Cohen et al., 2012). Likewise, there is evidence in humans showing that, in contrast, experiencing positive emotions may improve health. Pioneering researchers suggest that there are health benefits in humans of humor and laughter as

1. World Health Organization (https://apps.who.int/aboutwho/en/definition.html).

positive-emotions promoters, especially in coping with a diagnosis of cancer (Mahony et al., 2002). Although the scientific evidence for this claim is inconclusive (Martin, 2001), humor is being increasingly used as a means of reducing stress and making patients feel better (Penson et al., 2005). Obviously, there are no such studies on animals. Nevertheless, an increase in stress responses is often associated with an increased vulnerability to infectious, metabolic, and production diseases. It is logical to suggest, therefore, that giving increased opportunities for positive experiences might also have a positive effect on animal health. For instance, pigs given the opportunity to show successful adaptation by rewarded cognitive processes (i.e., cognitive enrichment) express a higher immune reactivity and recover more quickly from a standard biopsy (Ernst et al., 2006). Although psychological factors influence the immune system at any point in the lifespan, it has been shown in laboratory animals that the immaturity of a young infant's immune responses makes it more vulnerable, especially during the fetal and neonatal stages (Coe and Lubach, 2003). Various studies in humans show clearly that perinatal stress has a programming effect resulting in vulnerability to the development of chronic diseases in adulthood (Lupien et al., 2009). We can therefore hypothesize that proper welfare management of the young animal (either directly after birth or throughout the welfare state of the dam before birth) would improve its ability to cope with various forms of health challenges encountered not only during early postnatal life but also during its whole production life.

Although relatively recent, the concept of cognitive enrichment based on the evaluative abilities of the animals allows defining behavioral strategies that have the potential to sustainably improve animal welfare and maybe health. Such behavioral strategies such as animal-friendly housing should be easily implemented in farm husbandry and management as they are safe, relatively easy to implement, and economically affordable.

Conclusion

If we agree that farm animals are sentient and that they can more particularly feel emotions, then we should follow our representation of the sentient animals and behave toward them in the way that has been developed throughout this chapter. The starting point of the welfare of an animal is its emotions, which may or may not evolve into prolonged affective states (Veissier et al., 2012). We particularly emphasized in this chapter the cognitive aspects that can be useful to access emotions in animals. New conceptual frameworks based on cognitive sciences offer relevant tools to investigate positive affective states animals are capable of experiencing and to achieve real practical improvements in the welfare of the animals that we use for our own ends. Emotions are not merely determined by the characteristics of the challenges, but also by the personality of the individual animal. Avoiding the rearing of

overly vulnerable individuals through appropriate genetic selection and careful consideration of the maternal and early environment will help to improve the welfare of both animals and stockpeople. We saw that the implementation of cognitive challenges into animal housing (based on positive anticipation, positive contrast, and positive control) is a promising and practical approach to induce long-lasting positive effects on animal welfare and speculatively on animal health. Further research is necessary to fully understand how appraisal processes and ongoing cognitive coping influence the immune system before developing relevant behavioral strategies to enhance animal welfare and its health. Now, scientific information reported throughout this chapter should be enough convincing to promote livestock management practices, based on the animal's emotions, that ensure a better match between the animals' sentience, their own expectations, and their environment, to achieve a positive animal welfare.

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Chapter 4

Genetics and behavior during handling, restraint, and herding

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ABSTRACT
Different tests have been developed for evaluating the temperament of cattle, pigs, and sheep and some studies appear to have conflicting results. This may be due to confusion between the basic emotional systems of fear and separation distress (panic). Methods used for temperament tests can alter results such as how tightly an animal is restrained in a squeeze chute during temperament evaluation. Animals with a more reactive (fearful) temperament will exhibit greater agitated behavioral reactions when suddenly confronted with novel objects. Animals can be habituated to new things but learning is very specific. Habituation to one type of strange object may not transfer to other types of objects. Animals with smaller-diameter leg bones and slender bodies may be more reactive (fearful). Facial hair whorl position is related to a vigilant temperament, and it may be more evident in populations with more diverse genetic backgrounds.

Introduction

The primary objective of this chapter is to discuss the effects of genetics on the behavior of grazing animals during handling and restraint. Some of the species that will be covered are cattle, sheep, horses, and goats. Since the publication of the first and second editions in 1998 and 2014, there are numerous new research studies on how both genetic factors and early experience affect the behavior of herding animals. In the first edition, the primary emotional variable discussed was fear. Today, the concept of animal personality is becoming more popular (Wilson et al., 2019; Cabrera et al., 2021). The behavioral traits being studied are boldness versus shyness, exploration, sociality, and aggression. Neurobiological evidence shows that mammals have additional emotional systems that drive behavior (Morris et al., 2011; Panksepp, 1998). The brain mechanisms for psychological experiences exist

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in some of the most ancient regions of the brain and are similar in mice and people. Panksepp (2011) and Montag and Panksepp (2017) defined seven basic emotional circuits of mammalian brains.

One of the big problems now is that different terminology is being used to describe the same emotions. O'Malley et al. (2019) stated that comparisons between studies are difficult due to a lack of consistent terminology. Different words may be used for describing the same emotional trait. The following is an outline of the Panksepp (2011) basic emotional circuits with additional information on the newer terminology that is being used in some of the most recent studies.

FEAR—This emotion is triggered by a perceived threat. It is sometimes referred to as behavioral reactivity, behavioral agitation, startle response, or highly reactive temperament. More recent terminology for levels of fearfulness is Bold versus Shy (Goursot et al., 2019; Haigh et al., 2020).

PANIC—It is separation distress and sometimes it is referred to as social isolation stress or high social reinstatement behavior. The trait that O'Malley et al. (2019) calls activity in an open-field test may be PANIC stress. The animal may be reacting to being alone in the open field. Neave et al. (2020) reported that activity increased in socially isolated cattle. It is likely that the most common confusion in the scientific literature may be mixing up fear and separation distress.

SEEK—It would probably be similar to the exploration trait described by Cabrera et al. (2021) and Goursot et al. (2019).

RAGE—It is feelings of intense anger. In animals, it is referred to as aggressiveness (Cabrera et al., 2021). Rage motivates an animal to defend itself against a predator.

LUST—Sexual desire appetite.

CARE—It is maternal nurturing of the young and motivates behaviors such as grooming another animal. Some studies that use the term sociability may be measuring this trait (Wilson et al., 2019).

PLAY—Helps young animals learn new skills.

In this third edition, research studies will be viewed through the lens of Panksepp's core emotional systems. Highly variable results from many studies may be due to confusion between the different emotional systems. Fear and separation stress (PANIC) are controlled by two different emotional networks in the brain. The classic study by Faure and Mills (1998), Chapter 11, Genetic selection of poultry to improve welfare, on quail clearly shows that fear and separation distress motivated behaviors can be separated by selective breeding for different behavioral responses. See Chapter 1, Behavioral genetics and animal science, for a further discussion of the emotional systems and how they motivate behavior. During our careers, we have observed thousands of animals under many different conditions. In this chapter, we discuss our observations under field conditions and review research findings that affect behavior during handling, herding, or restraint.

Writing on the behavioral aspects of animal domestication for the *Quarterly Review of Biology*, Price (1984) stated:

> *It is difficult to generalize about the effects of domestication on either genetic or phenotypic variability because of different selection pressures on different traits and species. However, it is apparent that with respect to animal behavior, domestication has influenced the quantitative rather than the qualitative nature of the response.*

To put it in simpler words, domestication changes the intensity of behavioral response. For example, domesticated Norway rats are less cautious than their wild counterparts in a wide variety of situations (Price, 1998). Parsons (1988) further wrote that domestic animals are more stress resistant because they have been selected for a calm attitude toward man. In either event, a genetic predisposition to be fearful or calm interacts with early experience and learning in very complex ways. In 1998 in the first edition, the term temperament was used to refer to an animal's level of fearfulness, which was determined by both genetic and environmental factors. It is likely that the early studies of temperament in cattle mainly measured fear (Voisinet et al., 1997). Since the publication of the first edition, producers have been selecting beef cattle for a calmer temperament for over 20 years. Reducing high fearfulness may have unmasked other emotional traits. Recently researchers have discovered that two different standard cattle temperament tests are now measuring different behavioral traits (Bruno et al., 2016, 2018; Parham et al., 2019). Genetic influences on temperament interact with early experience and learning to shape adult patterns of behavior. During handling for veterinary or other husbandry procedures, fearfulness is a major determinant of an animal's behavior. In the following sections, we will discuss the interacting forces of genetics and experience. Some of the topics that will be covered are flocking behavior, herding behavior, flight zone, social behavior, attraction and reaction to novelty, effect of experiences on ease of handling, heritability of behavior, and sire effect. Some general principles for handling domestic animals will also be discussed. There will also be a discussion of the methods used to evaluate behavioral differences between individual animals of the same breed and differences between breeds.

Principles of herd behavior

Herding behaviors evolved as a defense against predators. A group of animals facing a predator will demonstrate herd behavior for protection. Herd animals find safety in numbers. One aspect of herd behavior often noted is that the herd is not completely interested in the protection of the group. Instead, self-interest is a primary motivator of the individual (Couzin & Krause, 2003; Hamilton, 1971). The selfish herd theory differed from the popular idea that the evolution of such social behavior was based on mutual

benefits to the population. Socially dominant herd animals, when they fear a predator work to get into the center of the herd so they are less vulnerable. Genetic factors are responsible for both species differences in herd behavior, and individual differences in how the animals react to the presence of predators or people. Domestic herding of cattle behavior may be different from wild herds of ungulates. Interestingly, people who work with wildlife have observed that African herding animals bunch together more tightly than similar animals in North America. This may be due to more predators such as lions, hyenas, and cheetahs. In domestic cattle, there are differences in exploration or SEEKING behavior between different breeds of cattle and within the same breed from different sires. Some Angus cattle are "go getters," and they will graze more widely than "laid back" cattle that stay near the water hole (Goodman et al., 2016). Some cattle are hill climbers and others prefer the flat ground and this may be related to genetic factors that have an effect on motivation and motivation (Bailey et al., 2015). Within a herd, certain cattle are leaders and others are followers (Moreno Garcia et al., 2020). It is likely that a leader is a high SEEK animal with lower levels of fear. To use the new personality terminology, the leader may be both bold and be high on the exploration traits.

Visual and auditory senses

With eyes positioned on the sides of the head, herd animals have a wide visual field (Hutson, 1993; Matthews, 1993; Prince, 1977). Panoramic vision enables animals to move together as a herd and constantly scan the surroundings for predators or other dangers. Grazing animals are dichromates and do not see the color red (Carroll et al., 2007; Jacobs et al., 1998). Dichromatic vision may also provide a greater sensitivity to visual contrast. Research on sheep and horses demonstrates that their visual system is designed for horizontal scanning while grazing (Saslow, 1999; Shinozaki & Hosaka, 2010). As herd animals graze on the grass with their heads down, elongated horizontal pupils allow them to scan the horizon for movement (possible predators) much better than round pupils. In addition, eye preferences for viewing certain stimuli are common in a wide range of species. The left and right sides of the brain are specialized to process information in different ways and to control different types of behavior (Rogers & Andrew, 2002). The left hemisphere (right eye) plays a role in assessing novelty and the role of the right hemisphere (left eye) in processing negative emotional responses (De Boyer Des Roches et al., 2008; Farmer et al., 2010; Robins & Phillips, 2009). Cattle and pigs will use their right eye and left brain to recognize something that is positive (Goursot et al., 2019). An approaching strange person was more likely to be viewed with the left eye. They would switch eyes for viewing a familiar person (Robins et al., 2018). The basic principle of right brain processes negative things and the left brain orients toward

positive things may only be true for threats or a resource such as food. Social interactions are more complex. Giljov and Karenina (2019) found that in bison, the right hemisphere was used for social bonding and the left for aggression. Sheep that were separated from a group of sheep had more ear posture changes and a higher percentage of animals with forward ears than feeding sheep (Reefman et al., 2009). Basile et al. (2009) found a right-ear, left-hemisphere preference of the auditory response to neighbor versus stranger calls in horses.

When in flight, cattle, deer, and bison all raise their tails. Kiley-Worthington (1976) suggests that the raised tail serves as a signal of danger to the rest of the herd. Social signals that trigger fear represent a particular type of fear-producing stimuli (Boissy, 1995). Research across species highlights the critical role of social fear learning, and the neural mechanisms are beginning to be understood (Olsson & Phelps, 2007).

Herding animals orient and point both their eyes and ears in the direction of any novel sight or sound. Orienting responses alert the animal to possible danger, are accompanied by an internal state of nervous system arousal (Boissy, 1995; Davis, 1992; Rogan & LeDoux, 1996) and elicit responses modulated by the nucleus accumbens in the brain. The nucleus accumbens mediates both fearful (FEAR) motivation toward threats and appetitive (SEEKING) motivation for rewards (Faure et al., 2008; Reynolds & Berridge, 2008). When an animal orients toward a novel stimulus, it may freeze, stand completely still and watch (SEEKING) mode, or go into (FEAR) motivated antipredator mode. The authors speculate that during the orienting response when animals are watching, the nucleus accumbens remains in SEEKING mode. The nucleus accumbens in the brain acts as a biochemical switch and contains neural circuits that can turn on fearful behaviors or approach-seeking behaviors. Typical fear-motivated behaviors are fleeing, bunching closely together, or fighting when the animal is trapped or defending calves. Both genetic factors and experience affect the response in the nucleus accumbens to either fearful stimuli or the motivation for rewards. For instance, rodents subjected to loud rock music and bright lights were more fearful because the fear-generating zones in the nucleus accumbens have expanded (Reynolds & Berridge, 2008). Conversely, a preferred home environment (familiar, dark, quiet) caused appetitive-generating zones to expand.

Differences in the strength of herding behavior of cattle and sheep

The first author has observed that wild ungulates such as the American bison have stronger herding behavior compared to domestic cattle. Separation of a single animal from the herd will cause it to make an intense effort to rejoin its herdmates (Grandin, 1993). Possibly this behavior is driven by the

emotion of PANIC (separation distress). Domestic cattle become more difficult to handle and sort when they become agitated and engage in bunching or milling. Each animal will attempt to push itself into the middle of the group where it will be safe from predators. The strongest socially dominant animals end up in the middle of the milling herd. In their natural environments, prey species animals such as elk and deer spread apart when grazing a hillside, but at the first sign of danger group closer together and flee as a herd. Domestic cattle living in areas with a large number of predators, such as wolves, graze in tighter groups than cattle reared in areas free of predators (Joe Stookey, personal communication, 2012). Small domestic herding animals such as sheep display similar behavior. When herding animals sense danger, they tend to flock together. Sheep tend to be more reactive and herd together more tightly compared to cattle. Sheep are more vulnerable to predators than cattle, therefore bunch together very tightly to find safety in numbers. Bunching is the only defense sheep have against predators. Cattle often turn and fight. Small, nimble animals such as antelope rely on the ability to quickly flee. Following the reintroduction of wolves in the Yellowstone Park area, ranchers reported that Angus cattle are becoming extremely aggressive toward both domestic dogs and wild canines.

Syme and Elphick (1982) found vocal and jumper sheep were seldom the first animals to move through a handling facility. Grandin (1980b) made similar observations in cattle. The wildest and most difficult to handle cattle move through a handling race at the end of the group. This was demonstrated by Orihuela and Solano (1994) with cattle in slaughter plants. Animals at the end of the group took longer to move through the race. Tulloh (1961) reported that Brahman cattle flock more tightly together. Whateley et al. (1974), Schupe (1978), and Hutson (2019) also showed breed differences in the flocking behavior of sheep. Rambouillet flock tightly and Chevoits are more independent.

Social behavior and handling

When a herd is moving, social rank can determine an animal's position within the herd. Animals with high social rank seldom lead (Hedigar, 1955, 1968). Being a leader is a more dangerous position, so animals with a high social rank lag behind or remain in the safety of the middle of the herd. Social behavior also has an influence on learning. Boissy and LeNeindre (1990) found that in tasks where cattle had to press a plate to obtain a food reward, the presence of a companion facilitated learning. Cattle trained with companions learned quickly compared to isolated animals. This was probably due to less separation distress (PANIC). Kilgour and de Langen (1970) further showed that the presence of companions lowers the secretion of stress hormones in sheep. The ability to detect and respond to signs of fear and pain in other members of the herd has probably conferred a strong selective

advantage during evolution. Animals as diverse as primates, birds, mice, cats, and cows learn fear by observing other members of their species (Curio, 1988; Olsson et al., 2007). Munksgaard et al. (2001) found the behavior of dairy cows was influenced by observing other members of the herd being handled gently or aversively. In a test of recognition, Elliker (2007) trained sheep to approach photographs of sheep with a calm expression rather than those of sheep with a startled expression. Ear position was the main feature used by the sheep to make this distinction, rather than eye features.

Social learning, social fear learning, and social recognition are familiar to most people that handle or raise domestic cattle and horses. Horse trainers use calm-trained horses to help ease the fear of novelty in young inexperienced horses. For example, loading in a trailer for the first time, being ridden for the first time, or even the stress of weaning can be reduced by having a calm companion horse nearby. In Australia, tame "coacher" cattle are used to assist in gathering wild feral cattle (Roche, 1988). In the United States, tame "Judas" horses are used to lead wild (feral) horses into traps or corrals. After the horses are herded by helicopter into an area near the trap corrals, the "Judas" horse is then released leading the wild horses into the corrals. Fordyce (1987) used a few tame steers to facilitate training Brahman calves to novel handling procedures. Petherick et al. (2009) found that both good handling practices and increased exposure to people during feeding in a feedlot reduced fear of people. On "Old West" cattle drives, lead steers with a calm temperament were kept around and excitable leaders were destroyed (Harger, 1928). Excitable leaders caused stampedes.

Flight zone

The flight zone is the distance within which a person can approach an animal before it moves away. Herd animals usually turn and face a potential threat when it is outside of their flight zone, but when it enters the flight zone, the animal turns and moves away. Hedigar (1968) first described the flight zone principle in wild animals. Kilgour (1971) and Grandin (1980a,b) further described flight zone principles for domestic sheep and cattle and showed how working along on the edge of the animal's flight zone, both cattle and sheep can be moved easily (Fig. 4.1). Small, flighty herd animals such as deer and antelope have larger flight zones than domestic herding animals (Fig. 4.2). They also have higher stress hormone levels than large, heavy animals such as cattle or Cape buffalo (Morton et al., 1995).

Genetic factors influence individual flight zone sizes between animals of the same species or breed, and between individuals within a species or breed. Flight zone distance is also determined by the risk an animal perceives in a given circumstance. Threats associated with greater risk (speed, size, directness of approach) influence flight initiation distance. In a large group of

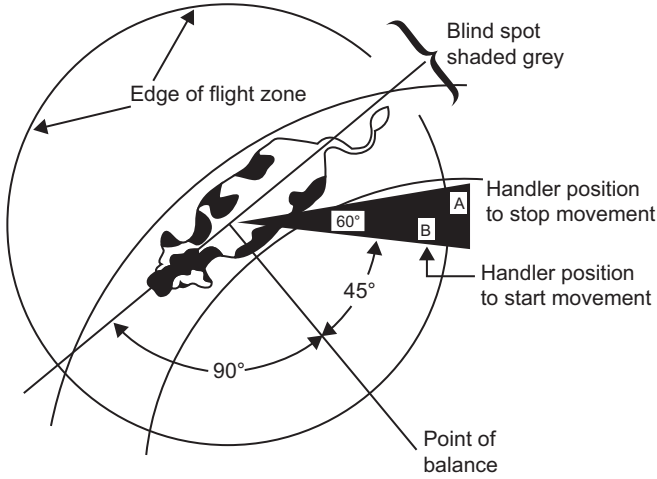


FIGURE 4.1 Flight zone diagram showing the correct position for a handler to move an animal.



FIGURE 4.2 Flight zone of a large flock of extensively raised sheep. The size of the flight zone varies depending on both genetic factors and previous experiences.

animals with similar genetics and previous handling experiences, most members of the group have similar flight distances, but a few individuals have either a very small or a very large flight zone. Flight zone size is also strongly affected by experience and learning. An animal that survives a close call with danger learns to be more wary of similar dangers in the future and increases its flight zone size accordingly. Furthermore, a completely tame or trained animal has no flight zone and will allow people to approach and

touch it (Price, 1984). Cattle that have people moving around them every day have small flight zones compared to genetically similar cattle raised on ranches in the mountains who seldom see people. Silva (2017) reported that crossbred Bos taurus calves handled with good handling and brushing had a long-term reduction of the size of their flight zone. Thus interactions between genetics and experience in shaping an animal's flight zone size can be complex.

Working the flight zone

Flight zone principles can be used by handlers to move single animals or large groups of animals. To move a single animal, the handler positions himself in relation to the animal's point of balance at the shoulder (Kilgour & Dalton, 1984). To move an animal forward, the handler is positioned behind the point of balance. To move the animal backward, the handler moves in front of the point of balance. When the handler is close to the animal, the point of balance is at the shoulder and tends to move toward the eye when the handler is further away (Grandin, 2019; Grandin & Deesing, 2008). When the handler moves rapidly the flight zone will get larger (Fukasawa, 2008). Fig. 4.1 illustrates the correct handler positions for moving an animal forward. Controlling the movements of a large herd while on foot, on horseback, or in a vehicle is done by alternately entering and withdrawing from the edge of the herd's collective flight zone. An entire group of animals can be induced to move forward by the handler moving inside the collective flight zone in the opposite direction of the desired movement. These positions work on all herd animals.

Dogs trained for herding use the principles of the flight zone and the same positions described above to control livestock movement. Coppinger and Coppinger (1993) suggest that dogs that herd livestock in this manner are displaying a natural hunting strategy used by wolves. Fortunately for the livestock, though, domestic dogs have been bred that do not attack or kill.

Measuring temperament in livestock

Livestock producers use temperament tests to select for calm cattle and sheep because they gain more weight and are more productive (Burrow and Dillon, 1997; Gavojdian et al., 2015; Toft et al., 2017; Voisinet et al., 1997). Extensive reviews have been written by Burdick et al. (2011), Dodd and Pitchford (2012), Forkman et al. (2007), Sebastian et al. (2011), Turner et al. (2011), and Benhajali et al. (2010). There is extensive evidence that behavioral variation in temperament may reflect underlying hormonal and neuroendocrine variation among individuals (Boissy, 1995). A variety of measures are used, and there is a need to associate each method with the correct core emotional system. Differentiation of the emotional system or systems being

tested may reduce many conflicting findings between studies. When temperament evaluation first started to become an industry practice in the mid- to late 1990s, it is likely that most testing methods assessed fear. Since the second edition in 2014, the first author did not realize how much genetic selection for calmness had changed cattle behavior. Our experiment station tried some new semen and bred some really nervous flighty Angus cattle. One steer was totally crazy compared to the rest of our Angus cattle. When the other steers stood quietly in the single file leadup race, this steer was so highly agitated that he attempted to break out. This steer reminded the first author of the cattle she handled over 20 years ago. All the cattle had been raised in the same environment. Due to over 20 years of selection for calmer cattle, other emotional traits may now be no longer masked by fear. This may be true for cattle in the United States and other countries where producers have intensively selected animals for a calm temperament. This may not be true in extensively raised cattle where there has been little or no selection for a calm temperament.

Tests originally designed to measure fearfulness in cattle

Restraint tests

It is likely that when restraint tests were first started in the 1990s, they mainly measured fearfulness (Voisinet et al., 1997). Since the publication of the second edition in 2014, it is now becoming clear that in the United States a restraint test and an exit speed test now measure different behavioral traits (Bruno et al., 2016, 2018; Parham et al., 2019). Years of selection for calmness may have greatly reduced fear-motivated resistance to being restrained. Some cattle may now be exhibiting anger-motivated behavior. The authors have both recently observed extremely calm cattle that stand quietly in a squeeze chute, and they exit slowly and calmly. Sometimes they may kick at people with deliberate carefully aimed kicks. The kicking behavior was not the flailing poorly aimed kicking of an animal overcome with fear. To conduct a restraint test, behavior is observed when the animal is held in either a single animal scale (Benhajali et al., 2010; Holl et al., 2010), a head gate (head stanchion) (Hoppe et al., 2010), or tightly held in a squeeze chute (Voisinet et al., 1997; Baszczak et al., 2006). One of the first chute scoring methods used a seven-point scale ranging from calm to highly agitated (Fordyce et al., 1988). Grandin (1994) used a five-point scale. A four-point scale is recommended because it eliminates a middle score. The scores are as follows: (1) calm; no movement; (2) intermittent movement; (3) continuous movement; and (4) violently struggling and attempting to escape (Grandin & Deesing, 2008). Hall et al. (2011) successfully used a five-point scale for assessing the temperament of pigs held loosely in a single animal scale. Restlessness and struggling are scored. Individual differences in

fearfulness and reactivity may be easier to differentiate when the animal is held loosely in a single animal scale or head gate. Sebastian et al. (2011) suggest replacing these subjective measurements with objective strain gauges that measure the force exerted against the head gate by the animal. Another possible objective measurement method would be a computer program used to analyze shifts in weight to measure sheep movement in a single animal scale (Horton & Miller, 2011). Many smart mobile telephones are equipped with accelerometers that can also be used to measure how much an animal shakes a restraint device (John Church, personal communication, 2012). Apps originally designed for monitoring earthquakes can be easily downloaded from the internet. A phone with this application could be used to measure shaking by taping it to the framework of the chute. Another possible automated method is using a computer algorithm to measure movement frequency from a video (Chen et al., 2020a,b). Tests that measure movement in a squeeze chute or scale may work poorly on purebred *Bos indicus* cattle such as Nelore if they have been subjected to a painful procedure, such as dehorning. *Bos indicus* may exhibit tonic immobility and freeze when they become extremely fearful. Tonic immobility is not likely to occur in crosses between *Bos indicus* and *Bos Taurus* and almost never occurs in the *Bos Taurus* (English or European) breeders of cattle. In female cattle, higher progesterone levels will make cattle calmer during a restraint test (Freitas-de-Melo et al., 2019).

Exit speed tests

Exit speed tests are also called flight speed tests or exit velocity tests. It is likely that these tests mainly measure fear. The speed an animal exits a squeeze chute or single animal scale is measured with either an electronic device (Burrow & Dillon, 1997) or by gaits such as walk, trot, and run (Baszczak et al., 2006; Lanier & Grandin, 2002). In some studies, exit speed tests have been shown to be more accurate compared to restraint tests (Café et al., 2010; Parham et al., 2019). This may be due to exit speed and restraint tests assessing different emotions such as fear versus anger. Several studies have shown that the score on an exit speed test had no relationship on cow or ewe protective behavior of her baby (Perez-Torres et al., 2014; Turner et al., 2013; Brown et al., 2015). It is likely that different emotional systems are involved. In studies where chute scoring was able to discriminate individual differences, the animals were restrained loosely. Hydraulic squeeze chutes that hold animals very tightly and restrict movement reduce the effectiveness of subjective scoring during restraint, making it difficult to differentiate between differences in behavior. In studies where restraint tests were effective for differentiating differences in behavior, researchers usually used looser restraint methods such as a single animal scale, a head gate or a manually (hand) operated squeeze chute (Benhajali et al., 2010; Holl et al., 2010;

Voisinet et al., 1997). Studies done by Baszczak et al. (2006) and Vetters et al. (2013) were conducted using hydraulic squeeze chutes that held cattle very tightly and exit speed scoring was shown to be more effective. Brazilian studies by Sant-Anna et al. (2012) report that subjective behavior scores were effective. It may be that the Brazilian restraint device held cattle more loosely than a hydraulic squeeze.

Exit speed scoring may not be accurate in Nelore or other purebred *Bos indicus* cattle if the animals freeze in response to handling stress. This is most likely to occur in Nelore that has been subjected to rough handling. Another factor that can confound exit speed tests is progesterone levels. Freitas-de-Melo et al. (2019) found that in Zebu-type cows, exit speed scores were slower if they were either pregnant or had a progesterone device.

Startle tests for cattle, sheep, and pigs

Startle tests mainly measure fear. In a startle test, an animal's reaction to a sudden novel stimulus is used. In sheep, a sudden blast of compressed air was used as a stimulus and the startle response was measured with accelerometers and visual assessment (Salvin et al., 2020). Some other examples of a sudden novel stimulus are the sudden opening of an umbrella, dropping a heavy chain, or a person stamping his feet. The reaction of pigs to a person suddenly stamping his foot was very effective for differentiating the behavior of different genetic lines of pigs (Lawrence et al., 1991). Startle tests are most appropriate for "one-time" temperament tests. Animals are likely to habituate quickly to repeated exposure to a sudden novel stimulus. When this test is conducted, it is important to separate the variables of a single animal being isolated from its reaction to the startle stimulus. Isolation may confound the results of a startle test that measures fearfulness if the wrong behavioral measures are used. Salvin et al. (2020) reported that when sheep were startled by a blast of air, an isolated sheep took three to four times longer to start eating than sheep with flockmates. Sheep will be stressed and repeatedly vocalize when a lone animal is separated from the flock (Hazard et al., 2016). Salvin et al. (2020) found that assessing the magnitude of the actual startle response was not affected by either isolation or the presence of other sheep. This study clearly shows that the reaction to the sudden loud stimulus and time to return to eating may be governed by different emotional systems.

Tests that measure separation distress, fear, and seeking

Isolation tests

Isolation tests measure PANIC (separation distress). In a typical isolation study, animals are separated from herd mates and placed alone in a pen.

Vocalization and locomotor agitation are scored. In sheep, Basile et al. (2009), Lansade et al. (2008), and Ligour et al. (2011) found that vocalization (bleats) was not correlated with a tendency to avoid or approach people. Isolating single sheep in an arena may be measuring two different emotional systems. The vocalization was probably measuring PANIC (separation distress) and the tendency to either approach or avoid people may be due to FEAR. In a heritability study of emotional reactivity, Boissy et al. (2005) exposed lambs to three challenging tests involving novelty, human contact, and social isolation. The highest heritability was for high bleats ($h2 = 0.48$) during social isolation. In horses, the frequency of vocalization (neighing) during isolation was stable over time (Lansade et al., 2008). The research also measured attraction toward other horses (gregariousness) and concluded that the trait of gregariousness is stable over a series of tests from 8 months to 2.5 years (Lansade et al., 2008). Possibly gregariousness and PANIC (separation distress) are separate but linked traits.

Open-field tests

Open-field tests may elicit a mix of emotional system responses: FEAR (a strong emotion caused by anticipation or awareness or danger), but may also measure SEEK (intensively energized to explore) and PANIC (separation distress). In an open-field test, a single animal is placed in an arena and its activity or the distance it walks is measured. Beausoleil et al. (2008) tested Merino sheep bred for either low or high activity in an open field. With a stationary person standing inside the arena, the more active genetic line walked around more, vocalized (bleated) more, and spent more time closely investigating the person in the arena. Analysis showed two emotional systems affecting behavior of the more active genetic line. The active genetic line was less fearful and more exploratory compared to the less active line. In a similar study using crossbred sheep that were not selected for high or low activity, Wolf et al. (2008) report the existence of considerable genetic variation in behavior. In addition, behavioral measures of locomotion/vocalization and proximity to the human are genetically and environmentally uncorrelated and represent independent measures of emotional state. The authors hypothesize that locomotion/vocalization in an open field is probably due to separation distress (PANIC).

Approach a novel object or novel person test

The tendency to approach or avoid a novel object or a person may be motivated by the emotion systems FEAR, SEEK, or PANIC. Previous experience and genetics strongly influence an animal's response to this type of test. In a typical novel object test, an object the animal has never encountered is placed in an arena and the time it takes to approach and/or touch

the object is measured. Kilgour et al. (2006) conclude that to assess individual differences in beef cattle to conditions of social isolation, human proximity, novelty, and restraint, the tests that best do this are the restraint test, the open-field test, the following test, flight time, the fear of humans test and flight distance. Recent studies are showing that two separate behavioral traits of fear and SEEK (exploration) are involved. Rozempolska-Rucinska et al. (2017) distinguished separate profiles of exploratory and fearful. Horses that are curious about novel objects were better at learning a new task (Christensen et al., 2021). Neave et al. (2020) stated that after puberty, the personality traits of boldness (low FEAR) and exploratory (SEEK) are stable. Social dominance is an independent trait, and it is not related to Boldness (low FEAR) or activity (Finkemeir et al., 2019). It is likely that their activity trait is PANIC because activity increased when a goat was socially isolated.

Pen testing

Pen testing measures FEAR, PANIC (separation distress), and Aggression. It can be conducted with either a single animal or a small group. In this test, cattle are placed in a small pen such as an auction arena and actively approached by a person. In France, this test is used by Limousin breeders to cull animals with a bad temperament. Cattle that charge people are not kept for breeding. Variations of the test are also used to determine the size of the animal's flight zone. Research by Paredes-Sanchez et al. (2020) clearly shows that pen scoring and exit speed measure different traits in Brahman cattle. There were significant differences in the genetic code (SNPs) associated with the two tests. Another variation of a pen test is the ease of moving a single sheep into a marked square on the floor (Bonato et al., 2021). This test was not able to differentiate different genetic lines of sheep.

Other behavioral indicators which may indicate genetic differences in fear

Eye white

The percentage of eye white showing in Black Angus cross beef cattle is highly correlated with exit speed score (Core et al., 2009). Eye white percentage likely measures negative emotions because anxiety-reducing drugs, such as diapazen, decrease the percentage of eye white that shows (Sandem et al., 2006). In this study, Norwegian Red dairy cattle were used. In Red Holstein and Brown Swiss, visible eye white did not differ between feeding and being restrained for hoof trimming (Gomez et al., 2018). The differences in the results may partly be due to differences in eye coloration.

Physiological measures

Cattle that become highly agitated during restraint or with high exit speeds often have higher levels of the stress hormone cortisol in the blood, and/or increased heart rate (Curley et al., 2004; King et al., 2006; Parham et al., 2021). Genetic factors influence physiological measures of handling stress. Zavy et al. (1992) found breed differences in cortisol (stress hormone) levels between *Bos taurus* and *Bos indicus* cattle. Furthermore, Zavy et al. (1992) found higher cortisol levels Brahman cross calves compared to crosses of Angus and Hereford. Stricklin et al. (1980) report Angus cattle having higher cortisol levels than Herefords. In Merino sheep, isolation stress increased plasma glucose 27% (Lennon et al., 2009). Pigs that squeal, jam in a chute, or were electric prodded had significantly elevated blood lactate (Edwards et al., 2010). Blood glucose and lactate are simple, economical tests but must be used IMMEDIATELY after an animal is subjected to restraint, isolation, or handling.

Syme and Elphick (1982) used a commercial heart-rate monitor to study heart rate and handling stress in merino sheep. The sheep were all neutered males raised on the same farm. Temperament classifications were (1) quiet, no response to being separated from the group, (2) jumpers, which attempted to jump out of the pen after being separated, and (3) vocalizers. Quiet sheep had lower heart rates compared to jumpers and vocalizers. Syme and Elphick (1982) may have been measuring separation distress (PANIC) instead of fear. McCann et al. (1988a,b) found a similar correlation between emotionality scores and heart rate in horses. Tests of emotionality included response to being herded, isolation, and being approached by people. Higher heart rates were recorded in horses having strong reactions to isolation and being approached by people.

Production traits

Restraint and exit speed tests in cattle, sheep, and other animals have been used to study temperament correlations with production traits. Animals with fearful, reactive temperaments gain less weight, have more dark cutters, and other meat quality problems (Café et al., 2010; Hall et al., 2011; Holl et al., 2010; Pajor et al., 2008; Voisinet et al., 1997). All the previously referenced studies are over 10 years old, and it is likely that fear may have been the major trait that was evaluated. A more recent study in Argentina with grazing cattle had less clear results. Meat quality and average daily gain were not affected by exit score (Della Rosa et al., 2018). Maybe the reason for no effect is that the cattle had already been selected for calmness. These cattle may have been slow, very slow, and very very slow. Another possibility is that the slowest speed cattle may have been lower on the SEEK trait and grazed less.

Relationship between fear and attraction to novelty

Highly reactive wild herd animals and highly reactive farm animals are both attracted to novelty and fearful of it (see Chapter 1: Behavioral genetics and animal science). Lawrence et al. (1991) found that reactive (fearful) pigs are most attracted to novelty (seeking). Pigs that were most likely to approach a novel bucket placed in their pen also showed the most intense startle responses to a person stamping his foot. In an open field test, highly reactive pigs also display behavior differences compared to nonreactive pigs. Dantzer and Mormede (1978) found that highly reactive Pectrain pigs walk around more in a novel arena compared to placid Large White Pigs. Pictrain pigs also learn an active avoidance task quicker than Large White pigs. William et al. (1964) also found breed differences in learning active avoidance tasks between excitable Hampshire piglets and placid Durocs. Hampshire piglets learn to avoid shocks quickly compared to Durocs. The breed effect was greater than the effect of individual differences between litters of the same breeds. Highly reactive pigs are vigilant and intensely aware of changes in their environment. Vigilance and reactivity may facilitate certain types of learning. The first author has observed that by confronting an animal with sudden novelty, the animal is more likely to show its true reactivity or level of fearfulness. Flight zone is possibly more affected by learning than reaction to a sudden novel stimulus that startles an animal. For example, Hereford cattle, a generally calm, placid breed, may have large flight zones when raised where they seldom see people. But even extensively raised Herefords seldom become highly agitated and struggle violently in a squeeze chute. On the other hand, tame halter broke individuals of Saler or Limousin cattle in the United States are much more likely to go berserk when suddenly confronted with the novelty of an auction ring, compared to extensively raised Herefords.

Animals with a fearful temperament have more violent reactions to a sudden novelty

Both authors have observed that the most reactive and fearful animals of a group are more sensitive to changes in the environment, and usually first to orient toward novel sights or sounds. Highly reactive herd animals constantly rotate their ear positions, raise their head quickly from the ground when grazing, excessively flick their tails, flinch when touched, or move away when approached by people. These behaviors are all signs of a highly vigilant reactive nervous system. Research studies and anecdotal observations suggest that responses to sudden placement in a novel environment, or reactions to sudden novel stimulus are very effective for identifying highly fearful animals. Cattle entering a novel auction ring and suddenly being confronted with people and noise is analogous to being placed in a novel

open-field test or suddenly stamping your foot in a group of pigs. Animals with genetically higher levels of nervous system reactivity and fearfulness are more likely to have violent reactions to a sudden novel stimulus (Grandin, 1997). In sheep, there is a relationship between high cortisol levels after slaughter and increased vocalization and vigilance during on-farm behavior tests (Diess et al., 2009). It is likely the sheep in this study were reacting to the sudden novelty of the slaughter plant.

Animal reaction to novelty is very specific

Animals do not generalize well between different objects or stimuli. Leiner and Fendt (2011) found that horses habituated to a stationary blue and white umbrella show fear to an unfamiliar orange tarp. After habituation training to the object, the fear-response to the object is specifically attenuated whereas the fear response to another object remains. Cattle with a very small flight zone when handled by a man on horseback have a much larger flight zone when moved the first time by a man on foot (Grandin & Deesing, 2008). Christensen et al. (2008) found that habituation to a novel object is stimulus specific. Horses tested with balls, boards, cones, or boxes were less fearful when all the objects were the same color. The first author hypothesizes that animals are sensory-based thinkers and remember objects as specific pictures (Grandin & Johnson, 2005). Further studies support our hypothesis. Megan Corgan, one of my graduate students, habituated young horses to a child's plastic playset by leading them by it multiple times (Corgan et al., 2020). When the playset was rotated 90 degrees, fully habituated horses were now reluctant to walk by it. The shape of the slide and the swing on the playset would be a different shape when it was rotated. The horses perceived it as something new.

Temperament and strength of emotional responses are highly heritable

Temperament in cattle is highly heritable. The heritability estimate of temperament in dairy cattle is 0.40 (O'Blesness et al., 1960), 0.53 (Dickson et al., 1970), and 0.45 (Sato, 1978). In beef cattle, the estimates are 0.40 (Shrode & Hammack, 1971) and 0.48−0.44 (Stricklin et al., 1980). In all these older studies, it is likely that fear was the major emotional trait that was assessed. The numbers of vocalizations in sheep isolated from flockmates during testing is highly heritable (Boissy et al., 2005). The trait of fear of humans is also highly heritable (0.38) (Hemsworth et al., 1990). Despite the abundance of evidence indicating that individual differences in temperament are genetically influenced, behavioral genetics research has more to offer the study of temperament than estimates of genetic influence. Environmental contributions to temperament are equally important, namely,

does temperament change across age and through experience? Intuitively, one might guess that as an animal matures and becomes more interactive with increasingly diverse environments, the role of genetic factors on fearfulness might diminish. Petherick et al. (2009) found that fear of people, flight speed, and the amount the cattle moved around a test arena were reduced by good handling. Exit speed also tends to decrease in older cattle that have been frequently handled (Parham et al., 2019). In another study, cattle that were more frequently rotated between pastures had slower exit speed scores (Ceballos et al., 2016).

Field observations on breed differences in cattle and sheep behavior

Cattle with Brahman genetics are generally considered "flighty" compared to breeds with British genetics such as Angus and Hereford. However, Brahman cattle are more inquisitive and investigate or follow a person or a dog. The tendency to follow a person is much greater in Brahman compared to British or European Continental breeds. Hohenboken (1987) found Brahmans can become extremely docile if handled gently. The first author has observed that Brahman cattle seek stroking from people more than Herefords. In Australia, a common practice used to move groups of Brahmans is allowing them to follow a person.

The authors hypothesize that *Bos Indicus* breeds may have stronger SEEKING and possibly stronger PANIC (separation distress) compared to *Bos Taurus* cattle. Murphey et al. (1980, 1981) discussed behavioral differences between several breeds in flight zone size, and the tendency to approach novel objects or a man lying on the ground. Breeds with the largest flight zones had the strongest tendency to approach novel objects. This observation is only true if animals are allowed to voluntarily approach novel objects. During forced movements when animals are being driven toward a novel object, just the opposite is true. The excitable individuals are more fearful and more likely to "spook" or balk. The second author has observed similar breed differences in the temperament of horses. High-strung breeds such as Arabians or Thoroughbreds take longer to habituate and stop fearing a novel situation such as new riding arenas, transport vehicles, or a strange person. Calmer, more placid animals habituate more easily to a novel stimulus.

Many well-documented examples report differences in temperament between different breeds of cattle. Stricklin et al. (1980) report that Herefords are the most docile British breed and Galloways are the most excitable. Early studies show that Angus and Angus cows are more temperamental than Herefords (Stricklin et al., 1980; Tulloh, 1961; Wagnon et al., 1966). Angus cattle dominated Hereford cattle in social rank even though they weighed less. Fordyce et al. (1988) found that Brahman cross cattle become more agitated during restraint than Shorthorns. Research by Voisinet

et al. (1997) had similar results. Braford, SimmentalRed Angus, Red Brangus, and Simmental × Brahman cattle became more agitated during restraint than Angus, Simmental × Red Angus, and Tarentais × Angus. Numerous studies show that English breeds such as Hereford or Angus are calmer than continental breeds such as Limousin or Brahma-cross cattle (Baszczak et al., 2006; Hoppe et al., 2010). Sheep breeds bred for meat, such as Suffolk or Texel, became more agitated in an auction ring compared to Merino and Corriedale breeds bred for wool (Dias Barbosa Silveira et al., 2010).

Problems with crossbred cattle

In 1993 the first author began to hear reports from cattle feeders in the United States about certain cattle unable to adapt to a feedlot. Most newly arrived cattle in feedlots adapt to vehicles and people within a few days. However, feedlot operators began to notice some crossbreeds with European Continental genetics Limousin or Saler were unable to adapt to the feedlot. Some animals had to be removed from the feedlot and returned to pasture. The breed associations select for calmer cattle. Limousin cattle today are on average are much calmer compared to herds in the early 1990s. Further observations by the first author suggest that behavior during handling or restraint of highly reactive European Continental crosses is different from the behavior of Brahman or Brahman-cross cattle. For example, Brahman cattle are seldom self-destructive when they become agitated. However, the first author has observed European Continental crosses more likely to injure themselves and panic during handling in squeeze chutes or truck-loading ramps. When Brahman cattle become extremely agitated they are more likely to lie down and become immobile. People in Brazil report similar tonic immobility behavior in *Bos indicus* Nelore cattle. The genetic differences in behavior became really obvious when different breeds of cattle are reared on the same pastures and handled together. A single dairy cow mixed in with Nelore cows had much calmer behavior (Grandin, 2019). Certain genetic lines of European Continental cattle are more excitable than British breeds (Grandin, 1994; Stricklin et al., 1980).

Sire and breed effects on ruminant behavior

Studies of sire effect on behavior clearly show differences in the behavior of the offspring of different sires that cannot be attributed to learning the behavior from the mother. Dickson et al. (1970) found that sire had a significant effect on milking parlor behavior and temperament of cows. Arave et al. (1974) showed that Holstein heifers from certain sires had higher activity levels and greater learning ability. Both ranchers and dairymen learn from practical experience that calves from certain sires are more nervous and

excitable. Torres-Hernandez and Hohenboken (1979) found a similar relationship in sheep. Ewes by Romney sires are more agitated when placed in a stressful test situation compared to ewes sired by Suffolk or Columbia ewes. Beef cows sired by cattle developed for use in the mountains graze more frequently on hilly, rugged terrain compared to cows sired by breeds such as Angus not selected to live in rugged terrain (VanWagoner et al., 2006). Bailey et al. (2015) found that cattle that were willing to climb hills and graze sloped pastures had genes associated with spatial memory, motivation, and movement. The breed of cattle has a significant effect on both distances walked and forage chosen on extensive pasture (Pauler et al., 2020).

Individual differences within a breed

In "Behavior and Evolution," Mayr (1958) wrote,

> The time has come to stress the existence of genetic differences in behavior, in view of the enormous amount of material the students of various forms of learning have accumulated on genetic variation in behavior. Striking individual differences have been described for predator–prey relationships, for the reactions of birds, or mimicking, or to warning colorations for child care among primates, and for maternal behavior in rats. It is generally agreed by observers that much of the individual difference is not affected by experience but remains essentially constant throughout the entire lifetime of the individual.

Individual differences in animal behavior have been widely discussed by Hirsh (1963), Scott and Fuller (1965, 1978), and Plomin (1990). Livestock animals show individual differences in behaviors similar to those found in laboratory animals and dogs. Cattle and sheep from the same breeding system definitely have individual differences in behavior (Schiller et al., 2020; Sebastian et al., 2011). Kerr and Wood-Gush (1985) investigated a relationship between individual behavioral differences and production measures in dairy cows. They propose that the behavior of dairy heifers in one context can be used to predict behavior in other situations. Furthermore, Kerr and Wood-Gush (1987) found that an individual dairy heifer's reaction to being touched by a human observer showed a consistent individual pattern that remained consistent for a 2-year period. In young horses from 9 to 22 months old, Visser et al. (2001) measured individual variation and consistency of behavioral variables related to temperament. One out of four temperament traits, "flightiness" (novel object test), proved to be consistent over time.

Kilgour (1975) used the open-field test as an assessment of temperament in dairy cows. Le Neindre et al. (1995) documented individual differences in docility of Limousin cattle. In this study, cattle were separated from herd mates by a person working their flight zone. Individuals were considered aggressive if they lowered their heads or charged the handler. This test may have been measuring separation distress (PANIC) instead of aggression.

Murphey et al. (1980) found he could approach dairy breeds more closely than beef breeds. Individual differences also affect grazing behavior. In cattle with similar genetics, some individuals prefer to graze on steeper slopes compared to other individuals that prefer flatter ground (Bailey et al., 2004).

Grandin (1992) assessed the temperament of bulls during restraint in a squeeze chute four times at 30-day intervals and concluded that to obtain accurate temperament evaluations on individual animals, each animal should be scored more than once. Temperament scores were stable over time for the calmest individuals and stable over time for the most agitated individuals. A test conducted four times easily identified the extremely calm individuals versus the extremely agitated. Wild Bighorn sheep show a similar stability of temperament over time. Reale et al. (2000) reported that behavior during capture was similar during repeated captures.

Effect of pig genetics on behavior during handling

Using several different handling tests, Lawrence et al. (1991) compared two separate groups of modern hybrid Cotswold Landrace × Large White pigs. Both groups were reared under the same conditions, but differed in their behavior. The first author also observed differences in the behavior of the same genetic line of hybrid pigs on different farms. Pigs from one farm were calmer than pigs from another farm. The first author speculates that differences are due to differences in how the managers selected breeding stock and differences in handling. One farm had a scale that was difficult to read if a pig became highly agitated in it. This may have caused the manager to select calmer breeding stock.

Handling tests used by Lawrence et al. (1991) were as follows: (1) willingness to leave the home pens; (2) movement ease through a hallway; (3) response to a suddenly approaching human; (4) restraint-resistance using a snout snare; and (5) vocalization during restraint. With the exception of vocalization, all tests had a strong tendency to correlate. The measurements used by Lawrence et al. (1991) are all relevant to ease of handling on the farm. A more recent study (Borstel et al., 2018) recommends using farm-relevant measurements such as behavior during weighing and injections. The single-best predictor of temperament was a suddenly approaching human who stamped his foot. The response was scored on a five-point scale: (1) no response; (2) mild flinch; (3) animal backed away from the person; (4) animal backed away and squealed; and (5) animal attempted to escape. One advantage of the stomping boot startle test is that the startle (fear) response is not confounded by other factors such as isolation. Lawrence et al. (1991) concluded that the temperament differences between individuals were stable over time. Yoder et al. (2010) divided pigs into vocal, or not vocal categories while loading on a single animal scale. Landrace pigs vocalized compared to Chester Whites, Durocs, or

Yorkshires. Yorkshire pigs were also more difficult to load onto the scale. Breeders need to be careful when selecting for behavioral traits. Aggressive pigs with more lesions on the front of the body stood more calmly in the weigh scale (O'Malley et al., 2018). Within a breed, laterality will significantly affect pig behavior during both open-field tests and approaches to human tests (Goursot et al., 2019).

Lean, rapid-growing pigs are more excitable

Ultra-lean hybrid pigs with very little back fat became popular in the United States during the mid-1990s. They were selected for three traits. They were rapid growth, large loin eye size, and thin backfat. The first author has observed some genetic lines of lean hybrid pigs are much more excitable during handling compared to fatter breeds of pigs. She speculates this effect cannot be attributed entirely to early rearing environments. Striking differences in pig behavior occur in lean hybrids of different genetic backgrounds raised in the same building operated by the same people. Many pork producers have commented that some genetic lines of lean hybrids are more excitable than older crossbred pigs with large amounts of back fat. The differences in behavior were first noticed when producers changed breeding stocks. Lean hybrids are more reactive and more likely to squeal when touched. Research by Holl et al. (2010) helps confirm these observations. They found pigs with a docile calm temperament and more backfat grew more quickly. Leaner pigs in a composite population of Duroc, Large White, and Landrace became more agitated on a single animal scale. Pigs with a very excitable temperament are much more difficult to handle. Grandin (1994) observed that excitable pigs can be moved easily if the handler moves slowly and avoids sudden movements. Sudden movements cause excitable pigs to bunch together and pile up.

Lean hybrid pigs cause serious handling problems in slaughter plants that process 1000 pigs per hour on a single line. The reactive pigs balk more and are difficult to drive. Most large slaughter plants were forced to install a second complete handling and stunning systems to handle the lean hybrids at a rate of 500 pigs per hour in each system.

Behavior problems in crossbred beef cattle during the 1990s

Behavior problems with crossbred cattle have been observed by breeders. Bonsma (1975a,b) stated that Brahman crossbreds have more temperament problems than purebred Brahman. The first author has made similar observations in crossbreeds of Brahman and Hereford cattle. Compared to the purebred parents, crossbred calves are more difficult to halter break. The authors speculate that when Brahman is crossed with Hereford or Angus, emotional traits of either separation distress (PANIC) or CARING are reduced. In the

early 1990s, there were problems with very excitable beef cattle in the United States that were likely to become extremely agitated when suddenly confronted with the novel surroundings of an auction market or slaughter plant (Grandin, 1994). Problems with highly reactive animals are most evident in cattle breeds imported from Europe and crosses of these breeds. Observations in feedlots and slaughter plants by the first author indicate that crossbred animals are more likely to become agitated compared to purebred European Continental cattle. These cattle were introduced to extensive ranching operations in the United States during the late 1960s and early 1970s and came into favor when the drive for lean beef motivated producers to select for lean rapidly growing cattle.

An example of genetic selection for leanness that causes handling problems in a slaughter plant was observed by both authors. Three hundred highly excitable fine-boned heifers of Saler and British genetics were impossible to handle calmly. Some engaged in self-destructive behavior. One heifer tore off her front leg, and two others had ripped off pieces of a hoof. This occurred after they stepped in a crack between the loading ramp and the truck that caused them to react with instant panic. Cattle with a calmer temperament are more likely to stop and withdraw their foot from the crack. One heifer fell down and became so fearful that she thrashed around on the floor and was unable to make coordinated movements to get up. Another pounded her head against the side of the chute after she fell down. The heifers constantly bellowed and if touched they kicked with both back feet. When a plant employee leaned over the side of the race to encourage one to move, she responded by kicking with both back feet to a height of about 7 feet (2 m), narrowly missing his head. One hind foot caught the brim of his hard hat with such force that the hat flew high in the air. This type of extremely agitated behavior has a strong genetic basis. Cattle with calmer temperaments do not display such frenzied behavior, even when they originate from a remote ranch and have minimal previous contact with people. The first author has observed thousands of cattle move through this particular facility. In several years, she had never observed such berserk behavior. This type of behavior is similar to that of a high-strung horse. Compared to cattle, horses are more likely to become extremely fearful and injure themselves.

Animal size, body shape, coat color, and temperament

One source of variation in body shape is found in Bergmann's rule. This principle states that within a broadly distributed genus, species of larger size are found in colder environments, and species of smaller size are found in warmer regions. These differences are described by Goodwin (2002), and Moen (1973) identified a geographic cline (gradual change) that affects morphology and behavior. Individuals from the northern extent of the range

tend to be stockier—with adaptations for energy conservation in cold climate—and to have less reactive temperament. Individuals from the southern extent tend to be more gracile (slender) in build—with adaptations to heat dissipation in hot climate and a tendency to be more reactive. A clear example of this principle can be seen in differences between *Bos Tauras* (English and Continental breeds) and *Bos Indicus* (Brahman) cattle. The phenotypic variation is obvious in body shape and size, coat colors, and temperament.

Body type and temperament in domestic animals

Body shape in animals is related to temperament. Krushinski (1961) reported that slender, narrow-bodied dogs had increased excitability compared to "athletic wide-bodied" dogs. The narrow-bodied dogs were more fearful. Martins et al. (2009) used 11 body measurements in Braford cows and classified temperament using both a restraint test and an exit speed test. Animals of big body size performed better in the temperament tests compared to the groups with medium and small body sizes. The first author has observed that selecting pigs and cattle for lean meat and thin back fat has resulted in animals that are more reactive to sudden novelty. This idea is supported by Holl et al. (2010) who found that leaner pigs were more agitated on a weigh scale. When animals are selected for leanness and bulging muscles they tend to be less reactive than lean, smooth-bodied slender animals. However, both types of lean animals are probably more reactive than animals with more body fat. Holmes et al. (1972) found that double-muscled Angus cattle had more excitable temperament scores. The first author observed that Charolais cattle bred in Quebec, Canada, with heavy bones and muscular bodies are very calm at a noisy livestock auction compared to slender lean Charolais cattle bred in the United States. When we looked at the different body shapes and sizes of different breeds of pigs, cattle, and horses, the physique−temperament relationship was obvious. Lanier and Grandin (2002) found that beef steers with thicker, wider cannon bones exited a squeeze chute more slowly than steers with thinner bones. Chen et al. (2020a,b) have recently found a relationship between cannon bone circumference and ACTH levels in the blood. Lighter breeds of sheep have stronger flocking behavior and larger flight zones when confronted with threatening stimuli (Hansen et al., 2001). In this study, a stuffed lynx or bear on a trolley caused a bigger reaction than a rain poncho on a trolley.

Another example of the physique−temperament relationship is seen in highly prolific Chinese pigs. The sows have an abundance of fat and bear many offspring. These animals are very placid and nonreactive, whereas pigs carrying the porcine stress gene are slender, have low amounts of fat, and are more reactive. Pictrain pigs and the Chinese breeds are extreme examples of the relationship between temperament and body morphology.

Type and temperament in wild herd animals

Body size and shape of wild herd animals are also related to temperament. Highly reactive, delicate, lightweight antelope and deer survive in the wild by fleeing quickly from predators. Heavy animals like cattle and bison survive by either attacking or fleeing. However, one of the heaviest herding animals, the Cape buffalo, survives by attacking because it is too heavy to flee. Fine-boned, defenseless animals such as antelope have the most excitable temperaments. This is especially true if they live in environments with many predators. Small species of antelope are hypervigilant and equipped with large ears. Impala antelope are more vigilant than larger wildebeests. They will raise their heads more frequently when they are grazing (Testroote, 2018). This enables them to orient and localize faint sounds of danger. Any change in the environment results in instant orientation and possible flight. Larger, heavier animals are more placid but can be dangerous to handle in a confined space. They are more likely to attack if handlers enter their flight zone. In handling facilities, the American bison is one of the most dangerous animals. In the 1980s and 1990s, the first author has observed bison butt a fence with their heads up to 20 times. Bison have also been known to eviscerate a horse with their upward curving horns. They appear to be intermediate in the flight/fight survival strategy. Bison will flee from danger, but being fairly heavy, they are known to attack when unable to flee. In a variety of hoofed animals, Morton et al. (1995) measured the stress hormone cortisol. There is a big demand for bison meat in the United States, and this has resulted in the selection of calmer animals. Deer that are raised in New Zealand are now calmer and can be handled in open pens. This would not have been possible 30 years ago (Grandin, 2019). This research showed that large animals such as the Cape buffalo are less stressed by capture and restraint compared to flighty antelope species such as the nyala. When 18 different species of African animals were captured, the Cape buffalo was the only species in which cortisol levels did not rise.

Coat color and temperament

Agouti is the natural color of wild rats. Instead of being solid, each individual hair has three bands of color. The typical Agouti color is a rich chestnut with dark slate at the base of the hair. The genetics of Agouti is very simple. The Agouti has two alleles “A” Agouti, and “a” nonAgouti. Dominant “A” produces the banding of hair we recognize as Agouti. Recessive “a” produces solid colored hairs. Many coat color mutations appear in Agouti rats bred in captivity: albino, black, hooded, and others. In a population of rats bred in captivity for 35 generations, Keeler (1947) noted that some of these mutations were associated with varying degrees of tameness. Black rats were much tamer than the agoutis. They described the black rats as “Already tame

by nature. If very excited they may click their teeth, but are not apt to bite." They conclude that the strain of tame albino rat (homozygous for black and hooded), "was not domesticated by painstaking selection over long periods of time, but was modified in morphology principally by the introduction of three coat color genes [albino, hooded, and black], and in behavior principally by the (nonagouti) black gene." Breeding for the black nonagouti coat color in rats resulted in tamer, easier to handle rats (Keeler, 1942). In foxes, graded differences in temperament were found between foxes selected for different coat colors (Keeler, 1975; Keeler et al., 1968). Foxes that were all raised in the same wooded compound had flight zone differences that ranged from silvers (200 yards) to platinums (100−200 yards) and to ambers (3−100 yards). It is interesting to note that the foxes became progressively tamer as more and more mutant coat color genes were added. Tameness was also highly correlated with adrenal gland weights. The size of the adrenal gland decreased and the body weight increased as the foxes became tamer. The animals with the most mutant coat-color genes were the tamest.

Trut et al. (1997) selected wild agouti rats for tameness for over 30 generations. In the beginning of the experiment, white patches of hair began to appear on the rats and increased until about 73% of the rats had white bellies. Over time, the white patches on the piebald rats grew larger and larger. In sum, selection for tameness correlated with the frequency and extent of depigmentation.

Genetic selection for body traits has an effect on behavioral traits, and selection for behavioral traits leads to changes in body traits (Belyaev, 1979). Coat color and behavior are connected via a common biochemical synthesis pathway of the pigments determining color—the melanins—and the catecholamide group of neurotransmitters forming the basis of the information processing system (Hemmer, 1990). In artificial selection studies conducted for the US Department of agriculture on over 34,000 guinea pigs, Wright (1978) showed that selecting for different hair colors changed many other characteristics. This early research revealed patterns of associated changes in body shape, relative size of certain internal organs, and changes in temperament. In many cases, coat color can be used as a genetic marker for such traits (Keeler, 1975). Coat color is associated with differences in physiology, morphology, and behavior in foxes (Belyaev, 1979; Keeler et al., 1968; Trut, 1999), guinea pigs (Wright, 1978), Cocker Spaniel dogs (Perez-Guisado et al., 2006; Podberscek & Serpell, 1996), and cats (Kendall & Ley, 2006).

Both authors have heard reports from dairymen that white Holsteins are flighty and often more dangerous to handle than more pigmented cows. These casual observations made by dairymen may be explained partially by findings from studies on coat color−temperament relationships in other animals. Tozser et al. (2003) used a scale test and exit-speed scores to assess temperament in a group of Red and Black Angus bull calves. In this sample,

the red bulls were calmer than the black bulls in the temperament score and were found easier to handle by the Stockmen. Experiments on horses also demonstrate that chestnuts are more reactive than bays and dark-colored sheep and goats were more motile than white ones (in Hemmer, 1990).

Hair whorls

In humans, it is well known that hair whorl patterns form in the fetus at the same time the brain is forming (Smith & Gong, 1974). Hair whorl patterns are associated with handedness (Beaton & Mellor, 2007; Hatfield, 2006; Klar, 2003; Schmidt et al., 2008; Zarate & Zarate, 1991), hemispheric language dominance (Jansen et al., 2007; Weber et al., 2006), sexual preference (Hatfield, 2006; Klar, 2003), and schizophrenia (Alexander et al., 1992; Yousefi-Nooraie & Moratz-Hedjri, 2008). Scalp hair patterning is determined at 10−16 weeks of fetal life and is secondary to the growth and shape of tissues that underlie the fetal skin, especially the brain. Thus aberrant scalp hair patterning may be utilized as a clinical indicator of aberrant growth and/or shape of the early fetal brain before 16 weeks' gestation (Scott et al., 2005; Smith & Gong, 1974; Tirosh et al., 1987). Children with developmental disorders, such as Down's syndrome and Prader−Willi syndrome have a high incidence of abnormal scalp hair whorl patterns (Smith & Gong, 1973, 1974). Many body traits form very early in development at the same time the brain is forming.

Hair whorl patterns and temperament in horses were first observed hundreds of years ago in Arabia. In his work as a horse trainer, the second author observed that a "high" hair whorl on a horses' forehead, or those with two spiral hair whorls were more reactive and "high strung." Other horse trainers and veterinarians have observed a casual relationship between hair whorl patterns and temperament (Barker, 1990; Friedly, 1990; Tellington-Jones & Bruns, 1985). Many scientists believed these observations were rubbish and that horsemen were wrong. In the early 1990s, we set out to find if scientific evidence supports these observations. We decided to study hair whorls and temperament in cattle. Cattle and horses have similar hair whorl patterns on their foreheads (Figs. 4.3 and 4.4). It was easy to observe large numbers of cattle with similar genetics and handling experience. Cattle are not handled in the same way as horses, and their behavior is not confounded by the experience of being trained for riding. The survey was conducted at a commercial feedlot in Colorado. A total of 1500 cattle were temperament-ranked (with the rating scale described previously) while restrained in a hydraulic squeeze chute for vaccinations. The cattle originated from many different ranches. Seventy-two percent were European × British crosses and the rest were zebu crosses from Mexico. To prevent bias, temperament rankings were collected by one person standing to the side and behind the squeeze chute. From this position, he was unable to see the hair whorl. A

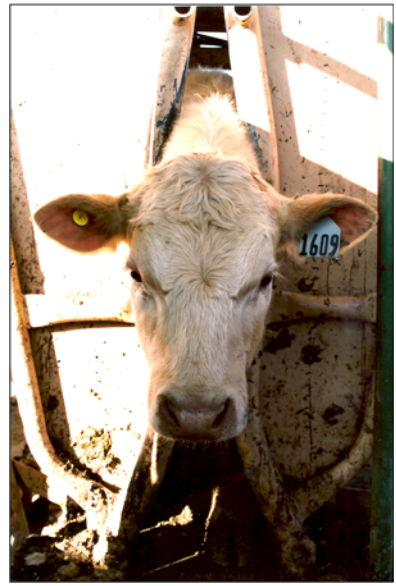


FIGURE 4.3 Hair whorl height is related to temperament in cattle. This animal has a hair whorl in the middle position between the eyes. Cattle with a spiral hair whorl below the eyes tend to be calmer.

second observer recorded hair whorl location from a position at the back of the squeeze chute. The hair whorl location was recorded before each animal entered the squeeze chute. We found that cattle with hair whorls above the eyes became significantly ($P = 0.001$) more agitated during restraint than cattle with hair whorls below the eyes (Grandin et al., 1995a). To our knowledge, this study was the first published evidence of a relationship between behavior and hair whorls.

Randle (1998) assessed the relationship between hair whorl position and temperament in 57 *Bos taurus* cattle in a post hoc investigation. Individuals were scored on 19 measures of personality, 14 of which related to temperament. Individuals with mid-whorls exhibited significantly greater flight distances to an unfamiliar human than did individuals with low whorls ($P < 0.01$). Cattle with high hair whorls above the eyes are more vigilant, excitable, and reactive (Bueno Ribeiro et al., 2012; Florcke et al., 2012; Martins et al., 2009; Aiequinq et al., 2020). Lanier et al. (2000) report that extensively raised beef cattle with high hair whorls are more likely to become agitated due to loud intermittent noise in an auction ring. Florcke et al. (2012) found mother cows with high hair whorls became aware of a strange approaching vehicle at a greater distance. In semiferal Konik horses, the height of a single hair whorl had no effect on startle response, but horses with elongated double hair whorls took significantly longer to approach a



FIGURE 4.4 This steer has a hair whorl above the eyes and this is related to a more reactive temperament. Since the late 1990s, American beef producers have selected cattle for a calmer temperament. Today, it is harder to find beef cattle with a hair whorl high above the eyes.

novel object (Gorecka et al., 2007). Possibly feral Konik horses have fewer variations in hair whorl height than more heterogeneous groups of domestic horses. Hair whorl patterns are highly heritable in horses (Gorecka et al., 2006), and Holstein cattle (Shirley et al., 2006).

The beef cattle in our first study had a higher percentage of hair whorls above the eyes compared to Holstein cows at a large commercial dairy (Grandin et al., 1995a; Tanner et al., 1994). Holsteins are much less reactive to novelty and aversive stimuli than beef cattle and there was no effect of hair whorl position on behavior (Shirley et al., 2006; Broucek et al., 2007). This is probably due to less variation in both genetics and hair whorl positions in Holsteins. In commercial feedlots, Holsteins are less likely to become excited during handling compared to beef cattle. In 2011 the second author collected hair whorl data on over 600 beef cattle in Nebraska. A small percentage of these cattle had high whorls compared to the data collected by Grandin et al. (1995a). This may be due to 15 years of beef industry programs designed to select for calm temperaments. Significant improvements in levels of fearfulness have been achieved by selecting populations against these traits. Selective breeding makes a group of animals more homogenous. This may possibly explain why Ebinghaus et al. (2016) also found no effect

on hair whorl height in German Black Pied cattle. Hair whorl position is likely to be more variable and has a significant relationship with behavior in groups of cattle or horses with more heterogeneous diverse genetic backgrounds.

Hair whorl height and vigilance

Animals with hair whorls located high on the forehead may have high nervous system reactivity, increased fearfulness and emotionality, highly acute senses, stronger orienting responses, and vigilance. A low hair whorl may be related to reduced vigilance and a reduced reactivity and fearfulness. Florcke et al. (2012) observed that Red Angus beef cows with high hair whorls were more vigilant when their newborn calf was threatened by an approaching vehicle. Cows looked up and oriented toward the vehicle when at a greater distance compared to cows with lower hair whorls. In Brazil, cattle with high hair whorls above the eye exited more rapidly from a restraining chute Bueno Ribeiro et al. (2012) and struggled more in a scale. Brazilian cattle have had much less intensive selection for temperament.

In cattle and horses, fine-boned, slender-bodied animals have a highly reactive temperament, a high hair whorl, and are more likely to have an explosive reaction when suddenly confronted with a novel stimulus that moves suddenly. Heavy-boned, muscular animals are more likely to have a calm temperament and a low hair whorl. Holstein dairy cows lack heavy muscling but fit the above criteria because they are heavy boned. We began to speculate about two separate genetic mechanisms influencing temperament after the first author visited the Lasater Beefmaster herd in 1996. Lasater's cattle have been closed to new genetics for 60 years and subjected to a unique set of selection pressures. Lasater (1972) selected his cattle using the natural principle of survival of the fittest. Heifers unable to give birth unassisted or to protect their calf from coyotes were culled. However, Lasater wanted commercially useful cattle so he also selected for temperament and carcass traits. The Beefmaster's breed is half Brahman, quarter Hereford, and quarter Shorthorn. Heifer's calves were selected as herd replacements if they willingly ate a food treat off a stick held by a seated person. Tom Lasater's son Dale explained how they selected for temperament. Instead of using sudden aversive novelty (such as restraint) as a temperament test, they assessed the temperament of hungry, newly weaned calves by sitting in the pen with them. Calves that failed to eat from a person's hand after 2 days were culled. When the herd was first started, about a quarter of the calves were culled. Today only 1% are culled for temperament (Dale Lasater, personal communication, 1996).

Lasater's selection criteria resulted in cows that are very protective of their calves, but extremely tame and seek contact with people. It is unusual to observe range cows that will come up and lick people and will stand still

while being scratched and petted. It is likely that they are selected for low FEAR, high CARE (maternal nurturing), and PANIC (separation distress). The appearance of these reddish-brown animals is striking. They are muscular and heavy boned with either high hair whorls on the forehead or no hair whorls.

Faure and Mills (Chapter 11: Genetic selection of poultry to improve welfare) demonstrated that the traits of fearfulness and social reinstatement are genetically separate. Social reinstatement and PANIC (separation distress) are probably the same emotional system. In Japanese quail, social reinstatement is defined as the tendency of an isolated bird to rejoin flockmates. In a wild population, it is likely that both fear and high social reinstatement would appear naturally in the same animal because this would improve survival. In domestic cattle, fearful animals bunch together tightly when excited. Tight bunching is probably motivated by high fear. Social reinstatement makes animals bond and is probably not motivated by fear. Faure and Mills showed that social reinstatement is separate from fear because they were able to select and breed both low-fear and high-social reinstatement birds and vice versa.

A second group of cattle similar to the Lasater Beefmasters's was observed by the first author during a trip to England in 2012. The cattle were Limousin × Devon cross cows that were very docile. They allowed strange people to approach them out on pasture, even though they had young calves. The cows were attentive to their calves and vocalized to call them as soon as they saw a strange tractor towing a trailer full of people entering the pasture. Most of the hair whorls on the cows were slightly above the eyes and no animals with extremely low whorls were observed. The cows were heavy boned yet extremely curious and quickly approached and touched the novel tractor. Even though heavy boned, their nervous system was vigilant. When I jumped off the trailer, most of the cows flinched but did not run away. The authors speculate that the combination of Devon genetics and Limousin had reduced FEAR, and produced animals that were high SEEKING, high PANIC (separation distress), and high CARE (maternal nurturing). Devons are bred to be docile and some farmers report they may push other cattle away from resources such as feed troughs. Possibly, Limoisin genetics helped to maintain the vigilance trait.

The effects of early experience on handling

Animals vary genetically in their susceptibility to environmental influences. Evidence indicates that some animals, such as those with emotional temperaments, are more vulnerable to the adverse effects of negative experiences. In fact, they are actually more vulnerable to *both* positive and negative experiences. Early rearing effects in cattle have been studied by Dellmeier et al. (1985). Calves were reared in individual stalls, which restricted movement.

When later tested in an open-field pen, the stall-raised calves were more energetic, had a greater level of activity, and ran around excessively compared to calves reared in group pens. Fordyce (1987) showed that adult cattle handled quietly as calves are quieter during handling compared to non-handled controls. Calves reared in an intensive system can also be sorted quickly compared to calves reared in a more extensive system (Boivin et al., 1992). Near weaning time, Boissy and Bouissou (1988) found that handling and contact with people improves the ease of handling in cattle. Early experiences will affect how animals react in the future to handling, restraint, or novel situations (Gonyou, 1993; Hemsworth et al., 1986).

The effects of early experience on handling are complex. Lensink et al. (2001) found that reactions of calves to handling depend on housing conditions and previous experience with humans. Calves were housed individually or in pairs. Half of the individually housed calves were stroked on the neck for 90 s each day, and half of the calves housed in pairs received stroking. Paired calves took more time to interact and interacted less with a person compared to calves housed individually. Calves that received additional contact with people interacted longer with a person than calves with minimal contact. It took more time and effort to load calves housed in pairs compared to individually housed calves, and less time to load calves that had additional contact with people. During loading, additional-contact calves had lower heart rates than minimal contact calves; whereas, during transport, pair-housed calves had lower heart rates than calves housed individually.

Lyons (1987) found that goats hand-reared by people, react less strongly to changes in the environment compared to goats raised on their dams. Differences in early rearing methods resulted in long-term, stable differences in temperament. Several studies show previous experience has a significant effect on an animal's flight zone size the physiological responses to handling and restraint (Boissy & Bouissou, 1988; Ewbank, 1993; Gonyou, 1993; Hastings et al., 1992; Ried & Mills, 1962). For example, both hand-reared cattle and hand-reared deer show lower cortisol levels during restraint compared to animals with less early contact with people (Boandle et al., 1989; Hastings et al., 1992). Hastings et al. (1992) also found "tame" hand-reared deer had lower cortisol stress hormone levels when restrained compared to deer raised by their mothers. Interestingly, both groups actively resisted restraint and vocalized. Compared to cattle with rough handling, Grandin (1984, 1987) concludes that cattle with gentle previous experience during handling will be calmer and easier to handle in the future. This principle applies to all domestic livestock, wildlife, even dogs.

Effects of environment and experience on pigs

Early environment effects on the behavior of pigs reared indoors have been studied by Grandin et al. (1987), Grandin (1989), and Beattie et al. (1995).

Pigs reared with assorted objects to play with, straw bedding, and daily petting were more willing to approach a novel object or novel person compared to pigs reared in barren pens. Environmental enrichment made the pigs calmer and less reactive, and they startled less when a person suddenly jerked upon the gate to their pens. Environmental enrichment also makes pigs more willing to walk through a single-file chute (Grandin, 1989; Pederson, 1992). Less prodding was required for the pigs from the "enriched" environment. Dudink et al. (2006) investigated whether the announcement of environmental enrichment, more than enrichment alone, could facilitate play behavior and reduce weaning stress. A sound cue was paired with environmental enrichment with a 30-s delay in which anticipatory behavior develops. Results indicate that announcement of enrichment and not enrichment alone increased play behavior, decreased aggression before and after weaning, and reduced the amount of injuries after weaning.

The first author has experience training market-weight pigs with excitable genetics to drive quietly at the slaughter plant. To facilitate handling and adaptability to commercial conditions, the training is done on the farm during the entire finishing time (fattening period). She found that by walking through the pens in a random pattern each day (10−15 s per pen) and training the pigs to move away in an orderly manner, the pigs learned to get up and flow around her as she walked through the pen. In this manner, pigs are taught to be driven quietly by people instead of learning to follow them. However, Grandin cautions the person walking the pens not to frighten the pigs. The idea is to teach pigs to become accustomed to people, to get up and move when people enter the pen, and not get upset and pile up. Walking the pens is especially beneficial for lean hybrids with nervous temperaments. Pigs differentiate between a person walking through their pens and a person walking in the aisle. This is why it is essential for a person to enter the pen and walk among the pigs. Further studies have shown that getting pigs accustomed to walking in the aisles and other handling procedures improved ease of handling (Geverink et al., 1998) and (Abbott et al., 1997). Pigs with practice moving through alleys and a ramp were easier to move through them in the future (Lewis et al., 2008).

Excellent memory of aversive experiences

Cattle, sheep, and other herd animals have excellent memories and remember aversive handling experiences (Grandin et al., 1986; Hutson, 1980, 1993; Pascoe, 1986). For example, Hutson (1980) found that sheep subjected to aversive procedures were reluctant to re-enter the test facility 6 months to a year later. Pascoe (1986) also found that dairy cows that had received shocks, remembered the shocks and showed higher heart rates when returned to the facility. Previous rough handling also makes dairy cows reluctant to enter a milking parlor (Seabrook, 1987). Cows previously handled in a gentle

manner had smaller flight zones and entered a milking parlor more quickly than cows handled in an aversive manner. It is extremely important that an animal's initial experience with a new handling facility be as positive as possible. If the animal's initial experience is aversive, it may become difficult to induce the animal to re-enter the facility (Grandin, 1993). The second author had an experience with a horse that hit its head the first time it backed out of a horse trailer. This one incident caused pain and fright and the horse remembered it. Following the incident, the horse learned to enter the trailer quietly, but during unloading, he continued to rush out of the trailer quickly. It was almost impossible to train the horse not to back out of the trailer suddenly.

Training highly reactive animals by introducing novelty gradually

Highly reactive animals can be conditioned to cooperate during veterinary procedures such as injections (Grandin et al., 1995b). To summarize the results of this study (discussed in detail in Chapter 1: Behavioral genetics and animal science), adult antelope at the Denver Zoo were trained to accept routine blood tests and their cortisol remained at baseline levels. This showed the antelope were not fearful (Grandin et al., 1996). The training was done in small, incremental steps over a period of weeks, and great care was taken to avoid triggering a massive flight reaction. For instance, when the antelope oriented and froze during any small change in the procedure, the procedure was stopped. Each day, new procedures were gradually introduced until the antelope habituated and no longer oriented. However, it is very important not to confuse taming effects on adult animals with changes that take place in the nervous system of young animals after handling. When the trained nyala were suddenly confronted with something not encountered during training, their true nervous system reactivity was displayed and they reacted with explosive fear reaction. Bongo antelope trained at the Denver Zoo had low (almost baseline) levels of cortisol when voluntarily standing in a box to have blood drawn (Grandin et al., 1996). Training experiments with pronghorn antelope show high specificity of their perception. Pronghorns trained to stand and receive an IV injection in the neck would remain calm and quiet because they had been carefully habituated to this procedure. When a student attempted to give an injection in the shoulder, they went berserk. An injection in the shoulder was novel and scary and an injection in the neck was familiar and safe (Grandin & Johnson, 2010).

In contrast to the highly reactive nyala or other reactive herd species, training placid animals such as Hereford cattle or Suffolk sheep to accept novel procedures can be done quickly and habituation to nonpainful procedures can occur even when the animals are pushed beyond the orienting response. However, if a highly reactive animal is trained too quickly and has

a massive fear reaction during the training, it may be very slow to habituate afterward. Highly reactive animals may become more fearful with each forced handling trial, whereas an animal with a more placid temperament may habituate and become less fearful. Grandin et al. (1994) found this in Angus × Hereford × Charolais × Simmental crossbred cattle that became increasingly agitated when they were repeatedly run through a squeeze chute on the same day. Animals need time to calm down between training trials and practical experience has shown that training trials should be spaced at least 24 h apart. Highly reactive cattle can be acclimated to repeated careful low-stress handling and restraint. Parham et al. (2019) found that both chute score (restraint) and exit score decreased each time they were handled and restrained in a squeeze chute.

During handling and driving in alleys, lean hybrid pigs have been observed to startle frequently and are more easily excited compared to fatter genetic lines of pigs (Grandin, 1994). The hybrids are highly reactive when a whip is cracked and raise their ears instantaneously in response to a sudden novel sound. Lanier et al. (1995) conducted experiments on genetically similar crossbred pigs with identical experiences and found individual differences in their adaptability to a forced novel swimming task. Some of the pigs readily adapted to a series of swimming tasks and others remained highly stressed. Some pigs had baseline epinephrine (adrenalin) levels after swimming, and others had high levels. In essence, the stressed pigs never adapted. To summarize, animals with a placid, calm temperament habituate more easily to forced nonpainful procedures than animals with a flighty, excitable temperament.

Innate nervous fearfulness or reaction to separation stress

A common question asked by cattle breeders in the United States is why cows with flighty European Continental genetics may be difficult to handle here in the United States and easy to handle in France. The answer: French calves are raised in close association with people and become tame. On ranches in the western United States, cows are normally handled only two or three times a year. Early intensive contact of calves with people in France produces calm, easy to handle adult animals. The highly reactive nature of some genetic lines of European Continental cattle may be not be displayed when they are handled in a quiet manner in close contact with people. In familiar surroundings, they may appear calm, but when suddenly confronted with novelty, they are more likely to have a violent reaction. Boissy et al. (1996) report similar findings in sheep. Romanov ewes originally bred for intensive conditions are stressed under extensive conditions due to a tendency to avoid humans. Further research has shown that gentling procedures designed to get young lambs accustomed to human contact had the greatest beneficial effect on the Gentile di Publia breed of sheep compared to the

Comisana breed (Caroprese et al., 2012). This study may indicate that the breed with the high separation distress (PANIC) benefitted the most. When a person was present in the test arena, the trained lambs vocalized less and had less attempts at jumping out (Caroprese et al., 2012). It is well-documented that early handling will make cattle and horses easy to handle (Boivin et al., 1992; Fordyce et al., 1985).

The managers of a bull test station reported two accounts of bulls that went berserk when brought to the station. The bulls charged fences and attacked the handlers, but afterward, the owner was able to walk them quietly into a trailer. They calmed down when they heard his familiar voice. In horses, both authors observed that the true temperament can be masked by learning. Some horses "blow up" when they are taken to a new place or exposed to a strange stimulus. For example, owners often teach their young horses to allow their feet to be handled by a horseshoer. The horse may learn to stand and accept this handling by the owner but when the farrier arrives for the first time, the horse may react violently. This may be due to the novelty of a new person. Some horses become fearful every time they are handled by a farrier. Owners often comment that this is "out of character" for the horse. When highly reactive animals are in familiar surroundings, they may be calm and their true temperament can be masked. They learn that certain sights and sounds are harmless. Recently, the owner of a high-strung horse commented, "He's 12 years old. You'd think he'd learn to get over the little things by now." Taming and training adult animals may reduce their reactions to specific handling procedures but it does not change their level of innate emotional reactivity. The level of emotional reactivity in an animal is like the level of water in a drinking glass. The level of water in the glass is influenced by both genetics and the environment during early life. Environmental factors can either raise or lower the initial level of water in the glass. Handling and contact with people early in life can reduce emotionality in an adult animal but it is almost impossible to manipulate the environment to such an extent that a highly reactive animal can become as calm as an animal that has calm genetics. For example, no amount of early handling can turn a high-strung Arab stallion into an "old plug" riding a stable horse. You might make him calmer, but he will never be as calm as a horse that is genetically calm.

Facilitating animal movement in handling facilities

An experienced dairy cow will walk over a drain grate in the milking parlor but young heifers often balk (Grandin, 1980a,b, 1996; Kilgour, 1971; Lynch & Alexander, 1973). Dairy cows can learn that drain grates and other things are harmless. However, animals being handled in an unfamiliar facility will balk and refuse to walk over puddles or drain grates. Shining reflections, shadows, and changes in flooring type will cause livestock to balk and refuse to move through a handling facility. This is especially problematic in places

such as auction yards or slaughter plants where the animals have no opportunity for learning. If leader animals are given a few minutes to investigate a shadow or puddle, they will usually step around or over it and other animals will follow (Hutson, 1980). Unfortunately, in a high-speed slaughter plant, there is not enough time to allow the leaders to investigate novel objects or areas of high contrast.

Emotionally reactive animals are often in a high state of nervous system arousal due to the novelty of the situation, which makes them more vigilant and likely to balk at small distractions in alleys and races that draw their attention. A jiggling chain, people up ahead, a sparkling reflection on a piece of metal, or air drafts and hissing exhausts blowing in their faces can all cause the animals to stop (Grandin, 1996; Grandin & Deesing, 2008; Tanida et al., 1996). More placid animals will usually hesitate and look at small distractions, but are more likely to keep moving. However, an area of high contrast or something that looks out of place is more likely to cause balking in nervous animals. In five different slaughter plants, the removal of all distractions such as sparkling reflections, air drafts, and the presence of people up ahead made it possible to greatly reduce the use of electric prods (Grandin, 1996; Grandin & Cockram, 2020; Grandin & Deesing, 2008).

Genetic effects on handling facility design

The first author has observed that the temperamental differences between Brahman breeds (*Bos Indicus*) and European Continental breeds (*Bos Tauras*) have had a substantial influence on the design of cattle handling facilities in the United States. These differences in design evolved over many years because ranchers used what worked. For example, in the northern United States, when cattle are being sorted into groups by sex or weight, the mostly placid British cattle are sorted in 3.5−5-m-wide alleys by people moving among them either on foot or on horseback. This method works because animals with a calm temperament can be easily separated from the group. Cattle with Brahman genetics are more excitable and have to be sorted in a 70-cm-wide, single-file race with gates at the end leading to separate pens. When Brahman cattle get excited, they engage in bunching behavior and herd tightly, which makes it difficult to sort out a single animal in a wide alley. Removing a single animal from the group is more difficult. It is much easier to sort cattle with an excitable temperament through a single-file chute. Hohenboken (1987) also commented that Brahmans behave differently in corrals and working facilities compared to *B. taurus* cattle.

Conclusion

Both genetic factors and the animal's rearing environment will determine how an animal will behave during handling. Animals with a highly reactive,

excitable temperament will become more fearful and agitated when confronted by sudden novelty compared to animals with a calm, placid temperament. Animals with a calm temperament will habituate more easily to new handling procedures. Stress levels will remain low in flighty excitable animals if new procedures are introduced very slowly. More recent studies support this concept. When handled in a low-stress manner, both fearful and low fear pigs will have similar 5-HT (Serotonin) levels in the amygdala (brain fear center). During stressful handling, the serotonin levels in the amygdala will decrease (Arroyo et al., 2016). An understanding of the influence of genetics on behavior will facilitate animal handling and training. Researchers are searching for easy-to-assess "objective indicator traits" (Chang et al., 2020). Behavior is complicated and an over simplified use of single indicator traits may lead to unintended consequences.

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Chapter 5

Reproductive and maternal behavior of livestock

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ABSTRACT
Reproductive and maternal behaviors of major livestock species are discussed in terms of their evolutionary origins, genetic influences, and heritability, and the effects on them of domestication, artificial selection, intensification, and environmental change.

General introduction

In ancient times, the health and well-being of the ancestors of today's livestock were subject to many challenges including predation, starvation, contagious diseases, and environmental extremes. Domestication has reduced the risk of exposure of animals to many of these threats while modifying the environment in which they are fed and bred, leading to lowered selection pressures on a number of traits that ensured survival in the wild but have become less relevant with domestication. Those attributes which facilitated success in sexual competition or in evading predators are less important today as breeding management, shelter, and protection are assumed by humans. Although the benefits of this arrangement are evident, the potential cost includes the dilution or loss of innate animal behaviors. Such considerations are integral to the wider challenge of producing more animal products to meet increasing global demands using production systems that are socially acceptable, environmentally friendly, and economically viable. Rapid increases in livestock production intensification are placing unprecedented strains on animal behavior and welfare, as well as increasing pressures to better understand and mitigate potential adverse side effects. An optimal balance between human requirements and animal well-being requires integration of best knowledge of animal behavior, adaptability, and welfare implications with management and production

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systems. This will also require appropriate education and training of animal owners and managers before it is a reality.

The challenges in achieving an acceptable balance between animal and human requirements are complex and vary with different environments. Climate change, decreasing natural resources, and a burgeoning human population place increasing pressures on food availability, security, and sustainability, much of which is still dependent on livestock. Although we have witnessed impressive animal production gains in recent decades, these are tempered by reduced reproductive efficiency in several species, particularly in more developed economies. The reasons for this decline, some of which are discussed herein, include genetic manipulations, such as inbreeding and overselection for certain traits, misapplied assisted reproductive technologies (ARTs), mismanagement, and environmental considerations, as well as interactions between a number of these factors.

In this chapter, we expand upon a previous account of reproductive and maternal behaviors in livestock (Chenoweth et al., 2014) by focusing on potential adverse effects linked with domestication and environmental change and their genetic implications. Particular attention is given to sexual and maternal behavior in several livestock species in which good maternal traits and adequate estrous expression are essential for the sustainability of production enterprises.

Evolution and domestication

Domestication includes the collective effects of isolating animals from their wild counterparts and natural environments and substituting these with an environment in which humans control their breeding, care, and welfare: a process in which animal genotypes, phenotypes, physiology, and behavior have undergone alterations (Dugatkin, 2018).

When considered within the context of evolution, domestication is a recent phenomenon (Jensen, 2015) and one that has "succeeded" with relatively few species. Those that have been successfully domesticated possess commonalities in those traits that collectively comprise the "domestication syndrome" (Hammer, 1984), with these including behavioral plasticity (Mignon-Grasteau et al., 2005), gregariousness (Diamond, 1999), inherited docility or tameness (Dugatkin, 2018), altered reproductive patterns (Dugatkin, 2018) including the ability to reproduce in captivity (Mignon-Grasteau et al., 2005), nutritional flexibility, and precocious young (Diamond, 2002). In addition, reproduction under domesticated conditions is facilitated by traits such as male dominance, sexual signaling via movement and posture, and extra-pair mating (Price, 1999). From the maternal perspective, favorable domestication traits include good maternal ability and strong parent−young bonding. Although livestock domestication has conferred innumerable benefits for mankind, it has also advantaged livestock in a number of ways, including relative protection from

predators, improved nutrition, and prevention or treatment of maladies and injuries, with associated improvements in viability and reproductive success (Price, 1984). Linked with these benefits is the relaxation of natural selection pressures associated with predation and starvation, and increased emphasis upon artificial selection for preferred traits, such as food and fiber production, draught purposes, entertainment, or companionship. However, although a number of historical stressors might be less evident under the conditions of domestication, it should not be assumed that they no longer exist. Livestock today must still cope with many challenges, some of which are amplified by domestication, including parasitism, pathogenic disease, social competition, and environmental stressors (Jensen and Wright, 2014).

Domestication, in itself, is not regarded as being highly heritable (Mignon-Grasteau et al., 2005) although some traits that have facilitated domestication are more highly heritable (Jensen, 2015). It can be useful to consider domestication as a factorial that includes behavior and reproduction, each of which is subject to multiple genetic influences. This perspective allows us to understand domestication as an incremental, ongoing evolutionary process in which resultant changes are more quantitative than qualitative (Price, 1999): an approach supported by the results of studies on changes in gene frequencies between wild and domestic animals (Jensen, 2002a).

Livestock behavior

Behavior has been described as: "all observable processes by which an animal responds to perceived changes in the internal state of its body or in the external world" (Barnard, 2004). This definition emphasizes two aspects, that is, that behavior is influenced by many different traits, and that recognizing these requires informed observation. These considerations are central to the discipline of ethology, which promotes the study of animal behavior within natural environments, and which has been instrumental in increasing our understanding of the genetic basis of behavior (Jensen, 2006). They also illustrate why the expression of behavior is not governed by direct, simple genetic mechanisms, but rather by a complex interaction of neurophysiological and structural factors, such as hormones and proteins, each of which is a product of indirect genetic effects (Friedrich et al., 2015). Thus it is not surprising that only 10% of differences in the behavior of laboratory rodents were directly attributable to genes (Flint, 2003).

An important distinction should be made between instinctive, genetic, or innate behavior and those that reflect learning and experience. The first group represents behaviors that are genetically preprogrammed to predictably occur in response to particular stimuli. Another term used is that of intrinsic characteristics, which, despite being less well defined, identifies particular characteristics as being essential and defining.

The optimal approach for studying human impacts on behavior in domestic animals is to compare them with wild members of the same species. However, this is difficult in today's world where wild counterparts of livestock are lacking and often represented by surrogates in contrived environments (Hall, 1979; Lindsay, 1988), or by related wild animal species in captivity. Despite such obstacles, it is generally assumed that domestication has not resulted in significant changes to basic, fundamental livestock behaviors (Jensen, 2015) and that the primary behavioral needs of livestock are not very different from those of their distant ancestors. This assumption is based on observations such as the ease and rapidity with which domesticated animals, given the opportunity, can revert to the behavior of their wild counterparts; for example, when domestic pigs become feral (Jensen, 2002b). Support also comes from observations that many innate behaviors persist, even in intensive production environments, even though they are unnecessary or irrelevant within that context.

Although innate behaviors are considered to be "hard-wired" within the genetic framework of a species, their ability to undergo adaptation to domestication is also facilitated by a number of traits that are, themselves, heritable (Jensen and Wright, 2014). These are, however, being increasingly strained by the inexorable pursuit of increased production and intensification. Thus, it is perhaps disappointing that, despite our burgeoning understanding of behavioral genetics, especially from laboratory studies, the application of this knowledge is relatively slight in livestock breeding programs (Canario et al., 2012).

Reproductive behavior

Commonalities exist in the reproductive behaviors of livestock. These include hierarchical social groupings, polygyny, sexual promiscuity, female estrous cycles, signaling of receptivity by pheromonal and behavioral cues, such as posturing and female mounting, and altricial offspring (Chenoweth et al., 2014). Differences, however, occur in aspects such as group composition throughout the year, seasonal reproductive capabilities in some species that can vary with location, female solicitation behaviors, copulatory behaviors, and dam−neonate interactions. With domestication, however, a number of these innate behaviors have been either encouraged or suppressed, with consequences that vary with species and context. Overall, domesticated species are relatively resistant to the stress of confinement while being able to reproduce under a variety of conditions (Mignon-Grasteau et al., 2005).

Sexual behavior can be partitioned into courtship and mating activities. Livestock are mainly sexually promiscuous and breed indiscriminately, with either males or females exhibiting individual or proprietary breeding rights. Contrary to anecdotal reports there is little evidence to support the contention that "falling in love" to the exclusion of other sexual partners is a common

occurrence in livestock, although mating preferences in boars were suggested in one study (Tanida et al., 1989).

Male breeding strategies, or the methods by which males gain access to females for breeding, include establishing harems, pair bonding, and opportunistic (or "sneak") breeding (McDonnell, 2016). In some species, the establishment and defense of territories is an important aspect, although one which is not easy to maintain under domestication. Male, courtship (or precopulatory) behaviors aim to induce overt receptive behavior in females, and they include such actions as male goats urinating into their beards, boars "nosing" females (Eberhard, 1996), and bulls performing false mounts. Male camels exhibit rutting behavior that includes increased activity including aggression toward other individuals, particularly males. Dromedary males extrude a portion of the soft palate (the "dula") during rutting and also release copious black secretions from the poll gland which acts as an attractant for females (Tibary et al., 2014). In general, males tend to be hierarchical and will compete for receptive females, with success often rewarding those of higher social rank, which is often linked with being the oldest, largest, and strongest. However, this success may not completely exclude subordinate bulls or rams from female access. Male libido or serving capacity differs among individuals and has a genetic basis (Chenoweth, 1981; Landaeta-Hernández et al., 2001; Chenoweth et al., 2014), with a reported heritability estimate of 0.59 ± 0.16 for serving capacity in a study of 157 paternal half-sib bull groups in Australia (Blockey et al., 1978). It is maintained fairly consistently throughout the year, which contrasts with seasonal female cyclicity in species such as sheep, goats, and horses in temperate zones.

Copulatory behavior comprises a series of actions including mounting, intromission and ejaculation. Species variations occur, particularly in the latter two categories, and these are often associated with the type of penile structure possessed by the male (Dewsbury, 1972), which, in turn, have evolved in response to both natural and sexual selection (Brennan and Orbach, 2020) including the risk of predation and social structures. Copulatory behavior itself probably owes more to sexual selection than to natural selection (Brennan and Orbach, 2020). Intromission and ejaculation occur rapidly in males with fibro-elastic penises, such as ruminants and camelids, and are more prolonged in those with blood-vascular penile structures, such as horses and donkeys. Sexually satiated males can exhibit the "Coolidge effect" in which sexual interest and copulatory attempts are renewed upon presentation of a new sexual stimulus; a phenomenon that helps to increase the distribution of male genes, thus promoting male fitness as well as polygyny (Dewsbury, 1981). In turn, polygyny, whereby males breed with a number of different females, is a common feature of livestock mating systems, being regarded as an evolutionary trait that has favored domestication (Price, 1984) as it allows individual males of superior genetics to propagate these widely. It also means that fewer intact males need to be managed, thus benefitting management effort and potentially, human welfare.

Polygyny facilitates sexual selection as competition occurs between males to impregnate the greatest number of females. Those individuals that are most aggressive and coercive will monopolize most available females, although problems occur if the dominant male is infertile while monopolizing the female cohort (Fuller-Fowler and Jenkins, 1976). In turn, aggressive and coercive male behavior encourages females to congregate in protective groupings; a response that could also facilitate polygyny (Cassini, 2020). For a more comprehensive account of sexual behavior in male livestock, readers are referred to Chenoweth et al. (2014).

Elements of the immediate environment can inhibit male sexual activity, with boars being influenced by pen-type including structural details such as flooring, walls, and protruding drinkers (Hemsworth et al., 1989), and this being most evident with young postpuberal males. The removal of potential constraints, such as the presence of a dominant male(s) or close confinement, is also an important consideration in the pursuit of optimal male sexual responses (Lindsay et al., 1996; Hemsworth and Galloway, 2012; McDonnell, 2016). Breeding farms and semen collection centers attempt to avoid such distractions while employing various strategies to optimize male reproductive responses and sperm yields. These include enhanced environments (Levis and Reicks, 2005), sexual stimulation, for example, observing sexual activity, and sexual preparation, for example, false mounts (Amann and Almquist, 1976).

In female livestock, sexual behavior has been described in three phases, that is, attractivity, proceptivity, and receptivity (Beach, 1976). Individual females differ in their degree of proceptivity and attractivity, and this can influence the degree of male sexual interest and behavior (Dewsbury, 1981). Sexual behavior is primarily dictated by hormonal changes associated with the estrous cycle. These hormonal changes can be influenced by season in species such as sheep and horses, particularly in temperate zones, and also *Bos indicus* cattle in the tropics (Galina et al., 1996) where Zebu breeds and their crosses are more fertile when days are longer (Taylor et al., 1995; Randel, 1984, 1994; Landaeta-Hernandez et al., 2020). Water buffalo in the tropics also exhibit seasonal tendencies, having greater fertility when days are shorter (Singh et al., 2000; Barile, 2005). The expression of estrus can also be influenced by stress, pain, estrous synchronization regimes, and environmental effects (Landaeta-Hernández et al., 2002) that can include heat stress and endocrine modifiers in the diet. Social effects can be powerful (Orihuela, 2000; Landaeta-Hernández et al., 2004b) such as with the relationship between increased numbers of females and abnormally short ovarian cycles in cattle herds: an effect observed especially in younger, more inexperienced individuals and probably due to aggressive competition from more mature cows (Moberg, 1985). In pigs, increased overcrowding (1 m^2/gilt instead of 3 m^2/gilt) tends to suppress estrous in cyclic gilts in association with increased levels of plasma cortisol (Hemsworth et al., 1986).

Mares will often show estrous behavior during the anovulatory period, which is unusual among ungulates. This could be an evolutionary strategy to

keep the social structure intact, by ensuring that the stallion remains with his harem throughout the year (Crowell-Davis, 2007). In feral horses, where linear dominance hierarchies are established within small stable breeding groups, social ranking is associated with reproductive success as dominant mares foal earlier, and have shorter foaling intervals, than their subordinates (Curry et al., 2007).

Studies on intrasexual rivalries have mainly focused on those occurring between males with less attention being given to female interactions, possibly because they are less obvious (Stockley and Bro-Jørgensen, 2011). However, females also form hierarchies and compete for males, although this usually does not exclude subordinate females from mating. When females are both attractive and receptive to males, this is commonly referred to as "being in estrous," or "being in heat." As females progress through proceptivity, they signal their impending receptivity by exhibiting greater motor activity, approaching males more closely, vocalizing, mounting each other, exhibiting standing behavior (or immobility), and by pheromonal effects. Female ruminants will often form a "sexually active group" which detaches from the main herd or flock to seek out males (Sveberg et al., 2013). Members of this group often display mounting behavior with each other, which, in turn, attracts male attention and sexual interest. The pivotal role that females play in the mating process is evident in many species, including horses, where females actively stimulate males and dictate the timing of copulation (McDonnell, 2000). Under natural conditions, mares are familiar with the stallions in their harem and are attracted to a particular individual by factors such as coat color, age, and dominance rank (Heitor et al., 2006). With domestication, however, such choice is restricted and mares may like or dislike the nominated male to the extent that their proceptive and receptive behaviors are modified accordingly, presenting challenges to those managing horse breeding programs.

Pheromones and biostimulation

Pheromones are chemical compounds that are species-specific in both their structure and actions, which include gender, reproductive and social cues (Brennan and Zufall, 2006; Kelliher, 2007). They have been categorized as those that induce social (releaser pheromones) and endocrine responses (primer pheromones), and also allelomones, which affect other species (Brennan and Zufall, 2006; Tirindelli et al., 2009). While they have been extensively studied under laboratory conditions, their roles in livestock reproduction have not received as much attention. The term is of Greek origin, being loosely translated as "something which excites from a distance," and it was introduced to the modern scientific world by entomologists. In contrast to insects, however, transmission tends to be more proximate in livestock, occurring via both the main olfactory system and the accessory

olfactory system (Tirindelli et al., 2009), which is represented by the vomeronasal organ (VNO). In cattle, the VNO response is triggered by the direct transfer of fluid by the tongue to the opening of the incisive duct system, which forms part of the accessory olfactory system connecting with the VNO (Jacobs et al., 1980; Berardinelli and Joshi, 2007; Berardinelli and Tauck, 2007). In turn, the VNO contains chemoreceptor neurons that detect pheromones and transmit the relevant information to those areas of the brain that control reproductive and social behavior. This occurs via a restricted, privileged pathway, although the signal transduction mechanism to the brain is not yet fully understood. However, the upshot is that precursors of gonadotropin-releasing hormone (GnRH) are released from the brain to facilitate the required response.

The term, biostimulation, is used to describe a variety of male, or male-like, effects on female physiology, behavior, and reproductive status and has been reviewed in different domestic species by Chenoweth et al. (2014). Biostimulation has long been exploited as a management tool, in different livestock systems, such as with the "ram effect" in sheep and with puberty induction in gilts (Chenoweth et al., 2014). In beef cattle, biostimulation has been reported to lower female age at puberty (Izard and Vandenbergh, 1982; Roberson et al., 1987, 1991; Oliveira et al., 2009; Fiol et al., 2010), induce ovarian activity in prepubertal Brahman heifers (Bastidas et al., 1997), accelerate postpartum cyclicity in both *Bos taurus* and *Bos indicus* breeds (Chenoweth and Spitzer, 1995; Soto-Belloso et al., 1997; Landaeta-Hernandez et al., 2004a; Landaeta-Hernández et al., 2008), improve luteal cell functionality (Hornbuckle et al., 1995; Landaeta-Hernández et al., 2004b), as well as breeding performance in progestogen-synchronized females (Chenoweth and Lennon, 1984).

Less work has occurred with dairy cattle, perhaps because artificial insemination (AI) is much more commonly employed in the dairy industry, as is reliance on exogenous hormones and drugs. In addition, dairy cattle results have been less convincing than those obtained with beef cattle (Fulkerson, 1984; Shipka and Ellis, 1998, 1999; Izaguirre-Flores et al., 2007), with this discrepancy being attributed to management differences, study methods (i.e., sample size and insufficient male:female interactions), and the interactions of nutrition and high milk yield. Despite this, the current decline in dairy cattle fertility in concert with increasing social pressures to minimize the use of exogenous drugs and chemicals in livestock would encourage increased exploitation of less controversial approaches, such as biostimulation.

Many studies have been conducted to better understand and exploit biostimulation effects in livestock, including female responses to male exposure in sheep (Rosa and Bryant, 2002; Fabre-Nys et al., 2015), goats (Chemineau, 1987; Murata et al., 2014), pigs (Brooks and Cole, 1970; Mavrogenis and Robinson, 1976), buffaloes (Barile, 2005; Zaidi et al., 2020), and horses (Ginther, 1983).

In sheep, exposing transitional temperate-zone ewes to rams resulted in increased ovulation (Pearce and Oldham, 1988) and conception rates

(Wheaton et al., 1992), while the sudden introduction of rams induced behavioral estrous and ovulation in ewes of tropical hair breeds in Africa (Ngere and Dzakuma, 1975). The reversal of seasonal anestrus in temperate-zone ewes by exposure to sexually active rams occurred via stimulation of kisspeptin neurons to produce GnRH, ultimately inducing the pulsatile release of luteinizing hormone (De Bond et al., 2013; Fabre-Nys et al., 2015).

The induction of estrous behavior and ovulation by biostimulation also occurs in goats, that is, the "buck effect" (Ott et al., 1980; Chemineau, 1987), with determinants including the type of contact, duration of contact, previous separation, male libido, and sensorial cues from the male (Delgadillo et al., 2009), with the latter including odoriferous and behavioral cues (vocalization). An olfactory signal molecule (4-ethyl-octanal) that activates the GnRH pulse generator in goats was identified as the pheromonal signal (Murata et al., 2014), with this being androgen-dependent and produced in the sebaceous glands (Iwata et al., 2000).

In swine, the biostimulatory effect of the presence of the male (i.e., the "boar effect") can advance the age of puberty in gilts. In addition, greater synchrony of estrous periods occurred when gilts were exposed to a rotation of boars (Brooks and Cole, 1970). However, other factors, either individually or in combination with the boar presence, are also capable of inducing puberty in gilts (Signoret et al., 1990), with these including stressors such as commingling, relocation, and transport (Hughes et al., 1997). The effects of biostimulation in gilts are influenced by the age of the gilts (van Wettere et al., 2006; Amaral Filha et al., 2009), the frequency of boar exposure (Hughes, 1994b), and also boar libido, that is, gilts exposed to high-libido boars attained puberty earlier than those exposed to low-libido boars (Hughes, 1994a). Boars under 9-month old were less effective than older boars (Kirkwood et al., 1981; Zimmerman and Kopf, 1986), possibly because of their reduced capability to secrete 16-androstene pheromone in saliva. Although the underlying mechanisms for the "boar effect" are still not clear, some of the factors that influence its expression are discussed by Hughes et al. (1990).

In domestic buffalo, biostimulatory effects and their exploitation is of relatively recent research interest. However, it is a relevant consideration for the application of AI in this species, which is constrained by problems with estrous detection. Exposing buffalo females to bulls from day 3 postpartum led to a reduction of silent ovulations and higher conception rates (Barman et al., 2011). In another study, the use of a progestin-based estrous synchronization program (Ovsynch) in combination with bull exposure induced anestrus female buffaloes to display estrous and subsequently become pregnant (Kalwar et al., 2019). Similarly, anestrus females treated with a CIDR-based protocol in combination with exposure to males showed greater estrous responses and blood progesterone levels than did those without male exposure (Zaidi et al., 2020).

Bisotimulatory effects may be induced in different ways, including pheromonal action, visual, and auditory cues, mechanical stimulation (Chenoweth et al., 2014), and probably other mechanisms that are yet to be revealed. Similar effects can be induced also by both estrous and androgenized females (Signoret et al., 1982; Burns and Spitzer, 1992; Wright et al., 1994; Chenoweth et al., 2014), and a biostimulatory effect of female to male (reverse biostimulation) has also been described in bulls (Prka, 2016) as well as in rams, where the pheromonal stimulus from estrous ewes acted to increase sexual performance in rams and also helped to determine their choice of mating partner (Ungerfeld et al., 2006).

In turn, the effects of biostimulation may be modified by stress and social interactions in females, and male-related factors such as sex drive, age, dominance (Landaeta-Hernández et al., 2013) experience, and relative size, with a quantitative effect being suggested by several studies. In one, the relative response of anovular, primiparous cows was associated with the frequency and duration of bull exposure (Berardinelli and Tauck, 2007), and in another, the biostimulatory response in female sheep was influenced by the level of male sexual activity (Delgadillo et al., 2009). This last observation was reinforced by work with cattle (Berardinelli and Tauck, 2007), where the intensity of stimuli (i.e., their frequency, quantity, and duration) significantly influenced biostimulatory effects in females. In sheep, the responses of young ewes to males were improved if they had prior experience with mature rams (Martin et al., 2004).

There is still much to learn before full exploitation of biostimulation in livestock species is achieved (Delgadillo et al., 2009). For example, the effects of nutritional and social interactions are yet inadequately understood, as well as other factors that are still unknown. Impetus for increased research efforts comes from the consideration that this phenomenon offers an attractive alternative to the use of exogenous chemicals or physical interventions in improving livestock reproduction, productivity, and behavior (Martin, 1995; Rekwot et al., 2001; Martin et al., 2004). In addition to the reproductive applications, as described earlier, promising avenues for exploitation of biostimulation and pheromones include (a) reducing animal reactivity to stressors and (b) mitigating adverse behaviors that cause animal distress and related welfare concerns as well as economic loss, including mismothering, abnormal mounting behavior such as with "buller steers" (Blackshaw et al., 1997), excessive male−male mounting (Freitas-De-Melo et al., 2014; Fajardo and Ungerfeld, 2021), and tail-biting in pigs. Such possibilities are reflected in an upsurge of interest in this topic (Delgadillo et al., 2009), which would benefit from better knowledge in areas such as its genetic influences and interactions with photoperiod and nutrition (Martin et al., 2004).

Genetic considerations

Evolution is only possible when there is sufficient variation in traits within populations for natural or sexual selection to occur. The degree of response

to selection, whether natural or directed, is influenced by (a) the amount of phenotypic variation present in relevant traits that are available for selection, (b) their degree of heritability, and (c) the degree of selection pressure that can be exerted (Kirkpatrick, 1996). For a comprehensive review of the relationships between behavioral genetics and animal domestication, see Jensen and Wright (2014).

Fertility, in general, is not considered to be of high heritability in livestock, although traits linked with fertility can be more heritable. For example, although the heritability of male fertility is generally considered to be low, some related traits, such as scrotal circumference in bulls and boars, are relatively heritable, and has favorable genetic correlations with other positive male fertility traits such as semen quality (Chenoweth and McPherson, 2014). It is possible that these traits might be successfully incorporated into selection indices for fertility as could other, more indirect, measures known to be related to fertility. In female cattle, for example, these could include age at puberty, calving interval, days open, days to the first service, and feed efficiency.

In common with fertility, heritability estimates for behavioral traits in animals are relatively low. Although genetic mechanisms control the physical actions that result in behaviors, the relationships between behavioral expression and its genomic underpinnings are far from simple. This complexity was recognized earlier by Fuller and Thompson who stated, "It has been found repeatedly that no one genetic mechanism accounts exclusively for a particular kind of behavior." This would explain why relatively few individual genes have been identified as having major effects on temperament and reproductive behavior in a species such as cattle, as well as the relatively low heritability estimates of social and reproductive behaviors in this species (Adamczyk et al., 2013).

Evolution also occurs through mechanisms other than selection, such as genetic drift, mutations, migration, and recombination (Kirkpatrick, 1996) as well as epigenetics (Jensen and Wright, 2014). The latter describe chemical modifications that can affect the expression of genes, both sporadically and more permanently and that are environmentally sensitive. Knowledge of epigenetics allows us to better understand those interactions that can occur between environmental factors and the genome, especially in relation to adaptive, nonrandom behavioral responses. However, appropriate exploitation of epigenetics is constrained by doubts that a significant amount of heritable epigenetic variance exists in livestock populations (González-Recio et al., 2015).

It is evident that the physical environment plays a significant role by directly influencing phenotypic behaviors as well as by modifying the genome leading to longer term, intergenerational, effects. Complications can arise when genetic correlations and linkages occur with environmental factors as well as between different traits resulting in the phenotypic expression of both unfavorable and favorable effects (Grandin and Deesing, 2014). In

addition, widespread pleiotropic changes can be expressed following selection on the basis of single traits (Jensen and Wright, 2014).

Although a focus on desired traits and their genetic transmission is central to any discussion of genetic progress in livestock, it is also useful to consider selection against undesirable traits. The feasibility of this approach was demonstrated in early work in Florida, where the systematic culling of nonbreeding beef cattle females was linked with significant gains in calf crop (Warnick, 1967) following failure in achieving similar results by direct selection for fertility. Further support comes from the work of Koeck et al. (2014) in which selection against fertility disorders in Canadian Holstein dairy cows resulted in improvement of herd fertility.

Inbreeding

Inbreeding is commonly practiced in the domestic livestock industries, both intentionally and unintentionally (Jensen and Wright, 2014). The term applies to the mating of individuals that are more closely related to each other than would happen under natural conditions. Thus inbreeding to some degree is to be expected in domesticated livestock as their breeding populations are generally limited. In addition, exploitation of ARTs can accelerate the process, for example, in dairy cattle where AI using a small number of elite bulls is extensively employed (Soares et al., 2011). Although there are differing perspectives on the genetic mechanisms and outcomes of inbreeding (Jensen and Wright, 2014), it does reduce heterozygosity in those traits affected by directional dominance, as well as contributing to the loss of advantageous alleles by random genetic drift (Sonersson, 2002). The resultant increase in genetic homozygosity can lead to greater delineation between different lines while reducing genetic variability, which can be advantageous for some breeding program goals. When combined with technologies such as best-linear, unbiased-prediction, and artificial insemination (AI), both positive and negative traits can be widely disseminated (Jensen and Wright, 2014), with the latter including a number of genetic defects in purebred animals. Conversely, inbreeding can also be a useful tool that is often deliberately employed to emphasize desired traits associated with production or appearance, including the derivation of "new" breeds.

Inbreeding depression is a term used to describe the decrease in "fitness" or "vigor" which is often associated with inbreeding (Jensen and Wright, 2014). Inbreeding has a negative effect on behaviors such as courtship and parenting in animal models, although definitive studies are lacking in livestock. For example, Leroy (2014), in reviewing 57 studies on inbreeding depression in seven livestock species, observed that behavioral traits received relatively little attention. This is despite evidence that reproductive traits that have strong behavioral inputs, such as fertility, fecundity, and maternal effects on offspring, are depressed by inbreeding.

Many livestock breeding programs are influenced by inbreeding to a greater or lesser degree, and for varied reasons. In reality, producers must balance desired genetic progress against the potential adverse effects of inbreeding and the emergence of undesired characteristics. Strategies to minimize the adverse effects of inbreeding in livestock breeding programs, especially in smaller (≤ 50) populations, have been described by Sonersson (2002).

The compromise between positive and negative risk factors associated with inbreeding is apparent with horses. The sequencing of the genome in Thoroughbreds (Librado et al., 2016) reveals that most genes that underwent positive selection postdomestication involved the cardiac and circulatory systems as well as bone and limb development, reflecting the need to improve speed, stamina, and racing performance. The genomic cost of this artificial selection has been an increase in genetic disorders (Librado et al., 2016), as is also witnessed with dog breeds that have been subject to both inbreeding and unbalanced selection.

Unbalanced selection and overselection

The undertaking by humans of livestock selection decisions to promote "useful" traits can often lead to an imbalance with other traits and functionalities (Grandin, 1998; Grandin and Deesing, 2014). Overemphasis on production traits or certain physical characteristics can result in abnormal developments that compromise biological functions or even survival. For example, the intensive selection for traits such as muscle growth, milk yield, and egg production can lead to unwanted morphological physiological, immunological, and behavioral side effects (Rauw et al., 1998; Rauw, 2007). This is illustrated with beef cattle where selection for muscular hyperplasia (or "double-muscling") is associated with a number of problems, including dystocia (Ciepłoch et al., 2017), and also in dairy cattle where selection for high milk production is linked to decreased fertility (Berry et al., 2020). In pigs, selection for rapid muscle development results in an increased prevalence of "tail-biting" and related injuries (Beattie et al., 2005), and selection for high growth rate is associated with increased aggressive tendencies (Rhydmer and Canario, 2014).

Here it is relevant to also mention diseases and disorders that go hand in hand with increasing levels of production (Ott, 1996). The topic of "Production Diseases" in farm animals is of sufficient importance to warrant its own annual international conference, and its relevance is increasing in response to rapid gains in animal productivity worldwide in association with ill-defined boundaries between production demands and the biological requirements of animals.

High production demands in ruminants impose increased metabolic demands which, in turn, reduce the capabilities of individuals to cope with stressors (Frisch, 1982), such as pathogen challenges. Other functional needs,

such as those of reproduction or health in general, are underresourced due to the competing needs of production. An overemphasis on production goals strains the physiological limits of animals, leading to increasing levels of "production diseases," such as lameness and mastitis in dairy cattle, in concert with lowered fertility (Dobson et al., 2001; Lucy, 2001; Mulligan and Doherty, 2008) respiratory disease in beef cattle (Garcia et al., 2010), and metabolic disease in both. The cost-benefits of such demands are reflected in dairy cattle in the South-east of the United States, where high production levels are achieved under hot, humid conditions at the cost of low reproduction and short herd longevity (De Vries and Risco, 2005).

Assisted reproductive technologies

In this discussion, an ART is defined as "any technique that interferes with the normal biological pathways of reproductive-related events and/or structures in order to contribute to the establishment of pregnancy with the final goal of producing healthy offspring" (Velasquez, 2008). ARTs provide invaluable tools for achieving desired genetic outcomes in livestock, as well as adding to our knowledge and expertise in livestock reproduction. They can be categorized as those requiring relatively low technical input, for example, manual pregnancy diagnosis and AI, medium technical input, for example, estrous synchronization (with insemination either following the detection of estrous, or at a fixed time, i.e., FTAI) or, use of ultrasound and embryo transfer (ET) and high technical input, for example, ovum pick-up (OPU), in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), use of sexed semen, and in vitro production of embryos. In livestock, the most commonly used ARTs today are pregnancy diagnosis (manual, or via real-time ultrasound), AI (including using sexed semen), and ET, although there is increasing demand for those of high technical input such as IVF, ICSI, and OPU, with cloning also becoming more popular in some species (Walton, 2018). A number of these programs require extra handling of animals, particularly for some complex hormonal regimes in which multiple injections may be given.

The effectiveness of ARTs in disseminating favorable traits rapidly and widely, however, is also true for unfavorable traits, and also genetic faults especially if semen is used from males of unknown carrier status for genetic defects In addition, by avoiding those natural obstacles that reduce the chances of defective gametes being "successful," procedures such as IVF and ICSI can contribute to increased early embryonic loss and decreased fetal viability. AI can widely propagate genes from males of low sex drive or dubious natural service capability, as with horses where stallions used for ICSI are often valued individuals that are infertile, incapable of service, or have limited semen availability (Galli et al., 2016). In a similar vein, procedures such as ET are not uncommonly performed with subfertile females

which are of high genetic value, thus allowing poor female reproductive performance to be propagated. A related concern arises with programs in which estrous is induced pharmacologically, obviating the need to observe estrous and thus allowing poor cyclicity and estrous signaling to go undetected.

ARTs can promote the rapid dissemination of genetic faults, especially if they are present in sires which are in great demand for other traits, such as for production or physical appearance. Although a full account of candidate genetic defects is beyond the scope of this discussion, they can be obtained from sources such as state extension services, for example, Genetic Defects—Beef Cattle (extension.org). Some that can negatively affect livestock sexual behavior include penile frenuli, spastic paresis, herniation, double-muscling, and conformational leg defects leading to lameness.

Balancing resources

The theory of relative resource allocation assumes that, within a given context, animal selection will achieve an equilibrium whereby necessary resources are optimally balanced between the demands of different major functions such as breeding, physical growth, and maintenance (Beilharz et al., 1993; Van der Waaij et al., 2002). However, imbalances can be caused by unnatural pressures imposed by processes such as artificial selection in which emphasis on one area or trait can cause constraints in another which is competing for the same resources. For example, increased selection pressure for production, without corresponding increased resources, can lead to other functions being underresourced. Specialized, high production animals are considered to be particularly vulnerable as they find it difficult to adjust to lowered resources, leading to outcomes such as lowered fertility in high-producing dairy cows (Rauw et al., 1998; Mignon-Grasteau et al., 2005).

Maternal behavior

Those activities of the dam occurring around the time of birth and which facilitate the successful birth and survivability of offspring are described as maternal behaviors. These can include separation from the main group, shelter seeking and nest building, the birthing process itself, cleaning and stimulating the neonate to suckle, and establishment of the maternal-offspring bond. In ungulate dams, a distinction can be made between “follower” and “hider” patterns of maternal behavior, as described previously (Chenoweth et al., 2014), although some flexibility can occur between these categories.

Good maternal behavior is essential for the survival and propagation of species in the wild and should also be a very important consideration for the successful breeding management of livestock. Each species has developed its own routine for birthing and subsequently nurturing and bonding with the neonate. Although maternal behavior is considered to be innate, it can also

be modified by both learning and experience. This is advantageous as modern production systems place inordinate strains upon innate livestock mothering ability (Edwards, 1983) which increase with intensification. In addition, factors such as selection for growth that results in larger fetuses and subsequent birthing difficulties can lead to reduced neonatal bonding and suckling (Jensen et al., 2008).

Although it is often assumed that domestication has resulted in qualitative and quantitative declines in livestock maternal capabilities, there appear to be relatively few definitive studies to support this assumption (Mignon-Grasteau et al., 2005). Conversely, several studies that compared maternal behaviors of domestic pigs and wild cross-breeds found little difference between the two groups (Gustafsson et al., 1999a,b; Spinka et al., 2000). Domesticated sows invested more energy in the current litter compared with the crossbreds which appeared to conserve resources to invest in a future litter. Such relatively small differences suggested that maternal behavior in pigs is strongly embedded (Nowak et al., 2000).

Maternal effects are derived from both phenotypic and genotypic sources, although major challenges still exist in separating genetic and environmental influences on maternal behaviors and capabilities (Räsänen and Kruuk, 2007). Prenatal stress can affect both maternal and sexual behaviors (Evans et al., 2016) while disruptions to the perinatal environment can significantly affect adult behavior, with both having the potential of being transmitted intergenerationally via epigenetic mechanisms (Jensen, 2015).

The low heritability estimates that have been reported for maternal behavior suggest that it may be difficult to improve genetically, even though selection for mothering ability is feasible for pigs, at least under field conditions (Hellbrügge et al., 2008). In addition, differences in the expression of maternal behavior between wild and domesticated sheep (Lindsay, 1988), as well as between different breeds of both sheep and cattle (Le Neindre et al., 1998; Mignon-Grasteau et al., 2005) also suggest that selection for maternal behavior could be successful under certain conditions. Selection against factors associated with poor maternal ability could be an attractive alternative strategy, as previously suggested with fertility. For example, selection against fertility disorders such as retained placenta and metritis, which have shown moderate to high genetic correlations with maternal calving traits in Canadian Holstein cattle, could yield worthwhile progress (Koeck et al., 2014).

Handling, management, and experience also play important roles in the qualitative and quantitative expression of maternal behaviors. For example, estrous synchronization programs can result in offspring being born en masse and often within a relatively confined area: a scenario that impedes natural birthing behaviors and that can also lead to confusion in bonding and nursing due to the proximity of newly born offspring and their mothers. Other examples illustrating the effects of management and experience, respectively, include sows that had been subjected to significant human handling being

less sensitive to the distress calls of their piglets, and differences in maternal behavior between uniparous ewes and those multiparous ewes that had undergone cesarean section (Houpt, 2018).

Temperament

The temperament of animals is the external reflection of how they perceive and respond to different situations, and it is an important consideration for livestock selection and management. For example, in some countries, the milking temperament of dairy cattle is included in selection indices (Adamczyk et al., 2013), while temperament is widely recognized as an important economic consideration in beef cattle. Despite this, it is uncommon for it to be included in beef cattle selection indices, possibly because of a perceived conflict with selection for production traits, as well as difficulties in assessment (Friedrich et al., 2015).

Temperament embraces a number of different traits such as sociability, aggression, curiosity, and risk aversion, and strong relationships exist between temperament, behavior, and "fitness" (Réale et al., 2007), with the latter being a measure of evolutionary resilience and survival under specified conditions. Matching temperament with different production systems is intuitively attractive as a possible avenue for improving both animal production and welfare (Martin et al., 2004; Boissy et al., 2005; Réale et al., 2007; Orihuela, 2021). As relevant research on this approach is lacking (Martin and Kadokawa, 2006), it is perhaps timely for this to be remedied.

Similarly, with other complex traits, such as domestication, temperament is multidimensional and thus resistant to oversimplification in terms of its description or assessment (Réale et al., 2007). It is usually assessed indirectly in animals, often in terms of reactivity to a specified stressor or environmental challenge (Sutherland et al., 2012), with the observed reaction commonly being fear (Grignard et al., 2001; Sebastian et al., 2011). As fear itself is usually a transitory response, the tendency to show fear, or degree of fearfulness, when faced with standardized stimuli, is used as a major indicator of temperament in livestock (Houpt, 2018). For cattle, relevant observations include behavior in a confined space, such as a chute or crush, and avoidance behaviors such as flight time, or the speed of escape from restraint (Burrow et al., 1988). Flight time is widely used as an indirect measure of animal temperament as it can be rapidly assessed during routine management procedures and it has been linked with production traits. In sheep, temperament is often described in terms of the degree of fearfulness expressed in response to human handling/contact or novel situations. Other methods used to gauge livestock temperament include flight distance, vocalization, and physiological signs such as heart and respiration rate and agitation levels (Murphy et al., 1994; Blache and Ferguson, 2005; Dodd et al., 2012, Réale et al., 2007). Using such methods, the heritability of temperament in livestock is

generally reported as being low to moderate (Martin et al., 2004; Adamczyk et al., 2013; Plush et al., 2011; Zambra et al., 2014).

With sheep, the ewe's affinity to her lamb, agitation score, and flight time (Plush et al., 2011) were used to assess ewe mothering temperament (EMT). Although the estimated heritability of EMT was moderate (0.35 ± 0.02), lower estimates were obtained for flight time and agitation scores (0.12 ± 0.05 and 0.20 ± 0.05, respectively). In addition, the heritability of litter survival was reported as low (0.09 ± 0.01), and its genetic correlations with EMT, flight time, and agitation score were 0.18 ± 0.08, 0.09 ± 0.27, and 0.39 ± 0.18, respectively. It has been reported that ewes of calm temperament are better mothers than those that are more nervous (Murphy et al., 1994), registering about half as many lamb mortalities from birth to weaning (single bearers, calm 7% vs nervous 16%; twin bearers, calm 16% vs nervous 26%). Similarly, Martin et al. (2004) considered that the poor mothering ability of nervous ewes was the main factor associated with lamb mortality.

In Arabian mares, which are reported to have poor maternal tendencies (Houpt, 2002), a relationship was observed between temperament, assessed in terms of fearfulness, and maternal ability (Budzynska and Krupa, 2011). Here, the greater maternal ability was exhibited by those dams that were more fearless. Interestingly, in semiferal horses in Australia, maternal protectiveness was considered to be more focused on potential infanticide by stallions, than on the threat of predation by dingos (Watts et al., 2020).

Although aggression in dams is considered to be advantageous in protecting the young, and consequently a positive asset for maternal behavior, there is insufficient evidence to support this widely held belief. Conversely, it could be considered that selection for calmer temperaments in beef cows might reduce maternal protectiveness; a concern in areas containing predators such as wolves. Additionally, anecdotal reports suggest that very calm cows could have greater problems with mothering, including weak calves, maternal neglect, and calf starvation (Sime and Bangs, 2010). Temperament has been linked with maternal protective behavior in *Bos indicus* cattle (Pérez-Torres et al., 2014; Orihuela et al., 2020), as well as with reproductive indices such as pregnancy rate and calving intervals (Enriquez et al., 2021) and also ovulation rate in sheep (Hart et al., 2008). In Canada, reproductive performance was lower in excitable beef cows than in those which were calmer (Kasimanickam et al., 2014) while dairy heifers that remained calm during head-restraint, achieved higher first service/AI pregnancy rates than did those that made overt escape attempts (Kasimanickam et al., 2018). Similar observations have been reported on the effects of stress on beef cattle reproduction (e.g., Fernandez-Novo et al., 2020).

With cattle operations, high levels of female aggression can raise both animal and human welfare concerns (Le Neindre et al., 2002) that may be even higher for *Bos indicus* genotypes that have a reputation for being strongly protective of their calves. Despite such observations, however,

research is lacking on the relationships between temperament, aggression, and protective behavior in cattle, and it is unclear how selection for calmer temperaments and reduced aggressive tendencies might affect maternal traits (Müller and von Keyserlingk, 2006). In *Bos indicus* mothers, maternal protective tendencies decrease with time postpartum, with 90% showing such tendencies at 25 days and 40% at 120 days after birth (Pérez-Torres et al., 2014). In a more recent study (Enriquez et al., 2021), a maternal protection test score was applied to 30 nursing *Bos indicus* females at 25 days postpartum. Here, apart from the interesting observation that 40% of the cows failed to exhibit maternal protective behavior at this time, no relationship was observed between ovarian activity and estrous display in either cow described as protective (P) or nonprotective. However, significant correlations were obtained between the intensity of reaction to the dam to her own calf and estrous display (yes/no) as well as the time of estrous onset.

The location of facial hair whorls (HWs) in cattle is cited as an indirect marker for temperament (Grandin et al., 1995; Pérez-Torres et al., 2014). Animals with an HW placed higher on their forehead were more "vigilant" (Flörcke et al., 2012), as well as being more prone to agitation during restraint, than those with a lower placed HW, which showed less signs of disturbance in the company of unfamiliar humans (Randle, 1998). These studies raise the possibility that the forehead hair whorl patterns of cows may be used as an indicator of maternal vigilance or protective behavior. Further study is indicated on relationships between HW location and maternal tendencies in cattle, as well as in other livestock species.

Identifying a beneficial linkage between temperament and mothering ability is of considerable interest to livestock producers as it could permit indirect selection for offspring survival (Plush et al., 2011). Evidence in support of this approach comes from the studies of Bickell et al. (2009) in which temperament ("calm" or "nervous") affected both maternal behavior and lamb survival in Merino sheep, and Phocas et al. (2006) who positively linked cow docility with reproduction traits including calving rate and the age at first observed estrous. In another study with ewes the flight distance of dams, assessed at tagging time of lambs, was genetically correlated with *a* lamb survival rate at marking. In an Australian study, over 14,000 ewes representing 32 breeds were scored for maternal behavior (MBS), flight time (FT*S*), and agitation (ABTS). MBS had a repeatability of 0.24 ± 0.01 and a heritability estimate of 0.20 ± 02, with some breed variation occurring. Although correlations between these measures were insignificant, it was concluded that better maternal behavior was associated with a lower score for ABT, and a longer FT (Brown et al., 2016).

Early weaning and separation of dam from offspring

The bond that develops between mother and young in livestock species is mutual, emotional, and relatively long-lasting (Weary et al., 2008; Enríquez

et al., 2011). However, it requires favorable circumstances for its full expression. These circumstances vary with different species, but generally include a period of secluded, exclusive contact at and immediately following birth. Unfortunately, this is often difficult to achieve in modern livestock systems where it is common practice for offspring to be separated from their mothers at a relatively early stage, and for direct suckling to be replaced by artificial means of sustenance. This results in overall benefits for production, reproduction, and thus profitability, although it is not in accord with "normal" practice. In nature, weaning of livestock offspring is a gradual process with both milk output and nursing frequency decreasing over an extended period. In contrast, in modern dairies, calves are separated within hours of birth from their mothers and responsibility for nursing is assumed by humans. When such an abrupt, permanent separation is imposed upon mother and young, there are consequences, especially when this occurs at a very early age. In lambs, for example, premature separation adversely affects lamb development, with these effects being worse when the separation occurred at 2 days (artificial rearing) rather than at 3-month old (early weaning) (Napolitano et al., 2008). Separated offspring must suddenly adapt to new diets, housing, and social environments in conjunction with loss of maternal contact and support. In addition, they are often exposed to a new and different environment in which they are commingled with weaners from other locations and backgrounds.

These disruptions are not only socially and physiologically stressful, but they also increase the risks of pathogens transmission and disease occurrence. This scenario is encountered in beef cattle when weaning and separation are combined with stressors such as commingling, transport, and relocation, leading to levels of disease, particularly bovine respiratory disease that are of considerable economic and welfare concern (Johnson and Pendell, 2017). Despite this, a review of literature on the effects of early calf separation in Australian dairy cattle (Beaver et al., 2018) was inconclusive on the effects that this caused to the health or welfare of either the calf or dam. The amount of stress incurred by weaners can vary with breed type, temperament, age of weaning, type of weaning (total or partial), and the extent of the physical changes that are imposed. In the mother, weaning can cause temporary discomfort from milk retention, and anxiety caused by the removal of pleasurable experiences associated with the proximity of the offspring and related activities such as grooming and nursing (Newberry and Swanson, 2008). It is clear that an improved understanding of all adverse effects of forced early weaning would encourage greater efforts in their alleviation. Support for relevant research comes from findings in other species, for example rodents, in which early separation led to changes in the offspring including modified gene expression in the brain and altered behavioral responses to aversive environments (Weiss et al., 2011). As the practice of early weaning of livestock is widespread, and increasing

(Napolitano et al., 2008; Enríquez et al., 2011; Orihuela and Galina, 2019), it would appear that similar research in livestock species is overdue,

In cattle, strategies for alleviating separation-induced distress include delayed separation of dam and neonate, provision of high-quality maternal care before separation, antisuck devices (e.g., nose flaps, udder nets), partial (fence line), limited contact before full separation, and gradual introduction to new housing arrangements (Stěhulová et al., 2008; Newberry and Swanson, 2008; Enríquez et al., 2010; Johnsen et al., 2018; Orihuela, 2021). One study compared responses in beef calves between physical separation with some contact (Price et al., 2003), and full contact with suckling restricted by a physical device inserted in the nose (Haley et al., 2005). Both scenarios reduced overt behavioral responses in cows and calves and allowed subsequent complete separation to occur with few signs of stress (Von Keyserlingk and Weary, 2007). One form of partial weaning, temporary calf separation (TCS), is widely used as a management tool to induce postpartum cyclicity in the dam, although most relevant research has been done with *Bos taurus* cattle (Pérez-Torres et al., 2020). However, a recent study with *Bos indicus* cattle showed that TCS for 72 hours caused greater stress to calves, and of longer duration, than the use of nose flaps to restrict suckling (Pérez-Torres et al., 2020). These and other studies indicate that separate manipulation of two major elements of weaning, that is, milk transfer and the separation of dam and neonate can generate less overt stress than when they occur simultaneously (Von Keyserlingk and Weary, 2007; Johnsen et al., 2018).

The common practice of early weaning in the pig industry has encouraged studies on the effects of different ages of weaning on piglet behavior and production, with a useful review of earlier studies being provided by Worobec and Duncan (1997). It is evident from these studies that a point is reached in lowering the age of weaning whereby the disadvantages start to outweigh the advantages. For example, Worobec et al. (1999) observed that weaning piglets at 14 days old or less was associated with increased welfare concerns and reduced performance, while Weary et al. (1999) showed that significant distress associated with separation and frustrated suckling occurred when weaning occurred at less than 4-week old.

Genetic aspects of maternal behavior in selected livestock species

Pigs

Pig production economics are dependent upon fertility, prolificacy (or litter size), and numbers of piglets weaned per sow, as well as suitability for the relevant market and the cost of getting them to meet market requirements. In general, female pigs have undergone selection for reproductive traits, while males have been selected for production traits such as growth, feed conversion, and carcass characteristics, with both favorable and unfavorable genetic interactions occurring among these desired traits.

Once piglets are born, a number of factors are involved in ensuring their survival and successful rearing to weaning, many of which are associated with sow maternal capabilities. Intensively managed pigs have been subject to greater constraints upon their natural birthing and mothering roles than most other livestock species, raising questions about the potential effects of these constraints upon maternal behavior, and thus piglet survival. If this were so, then an attractive proposition would be to improve sow maternal behavior by genetic means, potentially restoring aspects that might have been lost during domestication (Van Nieuwamerongen et al., 2014). This could possibly include greater selection pressure for those maternal traits that achieve a high number of weaned piglets under commercial pig operation conditions (Appel et al., 2016). However, the lack of evidence for significant direct genetic effects on maternal behavior in sows, as discussed later, discourages this approach.

Relatively high losses of piglets are caused by sows, either by crushing or savaging. Efforts to reduce the risk of crushing have included the use of farrowing crates, although these restrict the movement of sows, compromising their natural behaviors, and raising welfare concerns. Loose housing systems can potentially alleviate these concerns, although they are themselves associated with problematic levels of piglet mortality (Damm et al., 2005; Houpt, 2018). The heritability of being crushed is estimated to be higher early in a piglet's life than later, implying that risk factors associated with management and the environment are more important than genetic factors for losses of older piglets due to crushing. However, as causes of piglet losses are generally lowly heritable, direct selection against piglet losses per sé may be relatively unrewarding (Hellbrügge et al., 2008).

As sow behavior contributes significantly to piglet mortalities, it could be possible to reduce losses by selection for improved sensitivity in sows to the proximity and relevant communications of piglets, as well as for lowered aggressive behavior in groups (Hellbrügge et al., 2008), including reduced tendencies for fighting and bullying (Lovendahl et al., 2005). A Swedish study found that performing aggressive acts was moderately heritable in sows, although the propensity to be a victim was apparently not heritable (Rhydmer and Canario, 2014). Another possible avenue for improvement could be the removal of sows which required farrowing assistance from the breeding group (Appel et al., 2016). As pain and lack of mobility of sows are both capable of increasing the risk of piglets being crushed, selection and management tools aimed at reducing sow lameness should also help to reduce crushing of piglets (Iida et al., 2020).

In pigs, maternal behavior appears to be relatively resistant to adaptation or change (Nowak et al., 2000). The low heritability recorded for maternal and farrowing behaviors as well as for the number of born and weaned pigs, and weak genetic correlations between these traits suggests that selection for maternal behavior in pigs may be unrewarding, at least under confinement conditions, although it may be more effective under field conditions

(Hellbrügge et al., 2008). Selection for traits such as udder quality (Engblom et al., 2007) and number of functional teats can lead to indirect improvement (Appel et al., 2016) as these traits influence both maternal capabilities and piglet survival and are moderately to highly heritable.

Cattle

Maternal traits contribute significantly to the success of both beef and dairy cattle operations (Von Keyserlingk and Weary, 2007). However, despite the worldwide economic and cultural importance of cattle, research on maternal traits in cattle has been somewhat neglected, resulting in a lack of selection tools for improvement. This is especially unfortunate as fertility is reportedly declining in both beef and dairy cattle. With beef cattle, this decline has been associated with decreased pregnancy and calving rates and prolonged calving intervals. In dairy cattle, it is linked with decreased first-service conception rates and increased culling rates for infertility (Espinoza et al., 2016).

In general, maternal behavior in cattle is generally considered to be of low-to-moderate heritability (Michenet et al., 2016); although differences have been observed between breed types (Michenet et al., 2016; Mignon-Grasteau et al., 2005) and parity status (Zuko and Jaja, 2020). It is possible that strong maternal tendencies are more valued in beef cattle, in which calf protective behavior can be more important for calf survival than it is in dairy cattle (Le Neindre, 1989; Buddenberg et al., 2011).

In modern dairy systems, innate maternal behaviors, such as seeking a secluded birthing site, are often thwarted by management systems in which cows are confined at high stocking densities. Here, the practice of isolating calving cows in a maternity pen was shown to be more stressful than group calving (Kovács et al., 2016). In addition, the common dairy practice of removing the calf shortly after birth means that maternal behavior is often a lesser consideration than in rangeland beef cows (Rørvang et al., 2018), as suggested by stronger maternal bonding observed in feral cows compared with those that were group-housed (Vitale et al., 1986). In turn, this could be associated with the increased possibility of confined females finding themselves with a different calf than their own during the critical bonding period immediately after birth; a risk compounded by intensive housing systems and batch-calving following synchronized breeding programs. In general, dairy cattle have less intense maternal tendencies than beef cattle, in which traditional cow-calf bonding practices are often retained, in association with protracted suckling (Le Neindre, 1989).

The provision of adequate milk to the newborn calf is one of the most obvious expressions of maternal behavior, with an important aspect being the time taken for the calf to commence suckling or latency to nurse. This has special significance as the early milk supply is rich in colostrum, a major source of immunoglobulins that are important for the development of

immunocompetency in the calf. These immunoglobulins are macromolecules that can only be easily absorbed by calves for a limited period (approximately 12 hours) after birth. Calves that commence suckling after this time are deprived of immunoglobulins and are less immunocompetent. Latency to nurse is influenced by different factors, being generally longer in dairy compared with beef cattle, and also in primiparous compared with multiparous females, although calves on older dairy cows may take longer to commence suckling due to difficulty in accessing the large teats and udders of their mothers. This can also be a consideration in *Bos indicus* (e.g., Guzerat) cattle where oversized or "boule" teats in dams can hinder suckling (Frisch, 1982; Chenoweth, 1994). Latency to nurse can also be extended by stress and trauma encountered by the dam or neonate, at birthing. For example, inappropriately timed obstetrical interventions were associated with increased stress in cows at parturition, resulting in delayed recovery including inhibition of early maternal behaviors (Kovács et al., 2016).

Sheep

The large variations encountered in sheep genotypes and their management are reflected in a variety of expressed maternal behaviors and related effects upon productivity. Welfare implications identified with poor sheep maternal behavior are probably most evident in those regions where sheep are managed under extensive conditions, such as in Australia, where harsh environments, presence of predators, and poor mothering ability in the predominant breed, that is, Merino, combine to cause high levels of lamb losses. These losses have been estimated to be from 20% to 35% overall in Australia, with twins being implicated in even higher losses (Hinch and Brien, 2014).

Knowledge of sheep maternal behavior genetics is still far from complete, as it is with other livestock species. This includes genetic linkages and correlations between different traits and their interactions with the environment. For example, poor maternal tendencies in Merino sheep may be linked with their intrinsic characteristics of gregariousness and ability to handle harsh environments. Thus selection for improved maternal ability could result in lessening their productivity under extensive conditions. In addition, altering intrinsic characteristics is discouraged by EFAB (2013), which states, in its Code of Good Practice for Farm Animal Breeding Reproduction Organizations, that they should "maintain" the intrinsic characteristics of domesticated species"

In an Australian sheep study, a maternal behavior score (MBS; range 1, poor to 5, good) employed with over 14,000 records from 32 breeds (Brown et al., 2016), was shown to be both moderately repeatable and heritable. The conclusion was that sheep maternal behavior could be improved by selection, even though progress might be relatively small (Brown et al., 2016). Difficult and prolonged birthing can cause inappropriate behavior responses from both the ewe and neonatal lamb (Darwish and Ashmawy, 2011), suggesting that both selections

against predisposing factors as well as effective, timely intervention could result in better lamb survival and improved welfare of both ewes and lambs.

As a generalization, lamb survival is of low heritability (Safari et al., 2005) as are also those maternal and offspring factors that directly affect it, indicating that selection for lamb survival per sé would be unrewarding. However, selection for attributes that indirectly affect lamb survival, such as higher reproduction rates in general (Fogarty, 1984; Ercanbrack and Knight, 1998; Cloete et al., 2004) or improved lamb survival (Cloete et al., 2005), could prove more rewarding.

The establishment of a strong ewe−lamb bond soon after birth is very important for lamb survival and growth, especially with multiple births (Alexander et al., 1983). Maternal behaviors of the ewe include licking the newborn, assisting it to find the udder, and vocalizing to reinforce bonding with the newborn (Dwyer, 2014). Although ewes tend to isolate themselves for these activities, this behavior can be affected by breed, production system, and degree of domestication (Dwyer and Lawrence, 2005). Despite these variations, even sheep housed indoors will attempt to "isolate" at the edge of their enclosure or use a cubicle if available (Rørvang et al., 2018).

Horses

In common with other livestock, heritability estimates for reproductive parameters in horses are low. However, it could be argued that equine reproduction, especially with certain breeds, is more likely to undergo manipulation by human intervention than occurs with other livestock species. Although reproductive data in horses is often based on small numbers, one study used 696,690 breeding records of eight Spanish horse populations with different breeding purposes (Gómez et al., 2020) to obtain heritability estimates for reproductive parameters ranging from $h^2 = 0.02-0.42$ for mares and $h^2 = 0.04-0.28$ for stallions. In Finland, the heritability estimates obtained for foaling rates were $h^2 = 0.34-037$ for Standardbred trotters and 0.55−0.98 for Finn-horses, with these being affected by factors such as type of mating (natural vs hand serving), level of inbreeding, and age of both the mare and stallion. In this study, the maternal genetic effect was estimated to be 4.7% for Standardbreds and 3.2% for Finn-horses (Sairanen et al., 2009).

Infanticide, in which a stallion aggressively attacks a foal sired by another, has been reported in horses (Feh and Munkhtuya, 2008) although it was not observed in one study of semiferal horses (Jaworska et al., 2020). For further information on foaling in mares, postpartum care, and related problems, readers are referred to a review by McDonnell (2012).

Environment and stress

The physical environment exerts significant effects upon livestock reproductive behaviors and responses, including the capability to achieve

homeothermy, breed seasonally, obtain adequate nutrition, and cope with related stressors. As behavior, in itself, is of low heritability (Flint, 2003), this emphasizes the importance of environmental, and genetic-environmental interactions in behavioral expression. In general, animals possess anatomical and physiological mechanisms that allow them to (1) cope with environmental challenges in the short term and (2) adapt to ongoing challenges. The important relationship between adaption and the environment is recognized by several definitions of adaptability, including (1) the ability to survive and reproduce within a particular environment (Prayaga and Henshall, 2005) and (2) the degree to which an organism, population, or species can adapt to a wide range of environments (Barker, 2009; Canario et al., 2012). Today, the ability of livestock to adapt to climate variations is of increasing interest for livestock breeders; firstly because of public perceptions of climate change (Crona et al., 2013) and secondly because breeding practices, such as inbreeding and overbreeding, have resulted in reduced capacity of livestock to cope with, and adapt to, climatic variations. This second consideration was foreshadowed by Lerner (1954) who observed that the relative homozygosity of inbreeding is associated with increased sensitivity to environmental stressors, and confirmed by Leroy (2014) who reported that inbred livestock individuals are particularly sensitive to environmental changes. The intensive selection for a limited number of traits was also linked with increased sensitivity to stressors in males of modern poultry breeds, which were considered to have reduced capabilities to adjust to novel environments (Väisänen and Jensen, 2003; Huff et al., 2007).

Stress represents the response of individuals to stressors, and it varies in type and degree with each individual (von Borell et al., 2007). However, stress can adversely affect reproduction by interfering with mechanisms that control both the intensity of estrous behavior and fertile oocyte production (Dobson et al., 2012). The list of livestock reproductive traits that are susceptible to stress is long and includes the expression of sexual behavior, length of the estrous cycle, ovulation rate, mating success, and embryo survival (Martin et al., 2004; von Borell et al., 2007). Stressors can be short or long term, with the former being less likely to have adverse reproductive effects, while sometimes being stimulatory (Liptrap and Raeside, 1983), such as with the induction of puberty in gilts. In addition, the ability of an individual to recognize or control a stressful situation can vary with the health, immune, and reproductive status of that individual. A review of different tests for the genetic assessment of the behavioral response to stressors of cattle, pig, poultry, and fish in production environments has been provided by Canario et al. (2012). This comprehensive account provides estimates of the heritability of behavioral responses to stressors, as well as genetic correlations (minimum/maximum) between the different responses.

The quality and quantity of nutrition that animals receive are of fundamental importance for all aspects of their reproductive life. The mechanisms involved, however, are more complex than those associated with direct

environmental effects as the reproductive organs have some degree of autonomy and flexibility in responding to blood-borne nutritional inputs. Levels of energy and protein in the diet, as well as a number of micronutrients, can have important effects on aspects of reproduction in livestock, including puberty, cyclicity, ovulation rates, sperm production, and male libido. Although nutrition, in general, is too vast and complex a subject to be adequately covered in a chapter such as this, several aspects that are considered to be relevant for the current discussion will be mentioned, such as the nutritional management of young breeding stock, overfeeding, and the effects of some micronutrients as well as toxic products.

For example, low energy and protein intakes have both been linked with delayed reproductive development as well as longer term impairment of spermatogenesis and libido in the male (Singh et al., 2018). Undernourished bulls may lack both agility and stamina, especially if muscle wasting occurs. Conversely, the overfeeding of ruminants, particularly with high levels of energy over the period that includes puberty, can adversely affect female puberty and cyclicity, and permanently impair male sperm production, semen quality, and libido (Chenoweth, 2005; Singh et al., 2018); for example, over-fat bulls have been reported as lacking in libido, stamina, and mating competency (Chenoweth, 2005). Many of the side effects associated with animal obesity are not fully understood, although human male obesity has been linked with reduced androgen and increased estrogen levels, erectile dysfunction, reduced libido, and depression of both quantitative and qualitative sperm production (Raad et al. 2017). An additional consideration is that epigenetic changes associated with excess adipose tissue may be transmissible across generations (King and Skinner, 2020).

The influence of micronutrients on cattle reproduction was reviewed by Amin et al. (2016), in which deficiencies in copper, manganese, zinc, molybdenum, and cobalt were linked with such outcomes as lowered female cyclicity and estrous expression, male libido, or both. Gossypol, found in a cottonseed meal (CSM), has been shown to reduce spermatogenesis in male ruminants as well as cause sperm defects (Chenoweth et al., 1994), In one study, Velásquez-Pereira et al. (1998) reported that Vitamin E countered these effects in young CSM treated bulls, while also maintaining their libido.

Various toxic agents including endocrine-disrupting chemicals are capable of modifying animal behaviors, including those associated with reproduction. Exposure can occur through drinking water, feedstuffs (including contamination with fungal agents), plants (native or introduced) and from pollution such as from agricultural spraying, construction products, and industrial runoffs. Parental and social behaviors are particularly susceptible to the effects of endocrine disruptors as they are strongly influenced by androgens and estrogens. Livestock generally accesses endocrine disruptors from plants (as phytoestrogens), mycotic invasion of feedstuffs, and chemical pollutants, with plant-based sources being most implicated with clinical problems (Magnusson and Persson, 2015).

Environmental effects on livestock also include those of pathogens that adversely affect reproductive behaviors, both short and long terms. Examples in cattle include infections with viruses such as Bovine Ephemeral Fever virus, which causes pain and lassitude in affected animals and Bovine Herpesvirus-1, which is linked with acute balanitis in bulls, while infection with a blood-borne protozoon, *Theileria orientalis*, has been reported to lower libido in Friesian bulls (Gibson et al., 2020). Here, it is of interest that increasing the degree of biodiversity, as represented by different behavioral phenotypes, reduces the ease with which pathogens are transmitted (Dizney and Dearing, 2016). Longer and warmer summers associated with climatic changes have also resulted in a more northerly transmission of the Bluetongue virus in Europe and the extended geographic range of biting insects and fruit bats, both of which can carry noxious livestock viruses, in Australia.

As climate change, including rising temperatures, continues to significantly affect livestock behavior, health, and productivity (Mader et al., 2009), it is necessary to adapt both genetics and management accordingly. This was recognized by Jensen et al. (2008) who observed that "a central challenge for the future will therefore be to find farming practices where the genotype and the environment harmonize." This has particular relevance for tropical regions where animal production falls short of its considerable potential as well as in meeting human requirements, and where knowledge and resources are lacking to implement appropriate solutions. It is hoped that rapid scientific progress in areas such as genetics and genomics will help to improve livestock selection and management to best complement different environments.

Selected areas of concern

Reduced male reproductive potential

In prehistory, male reproductive "success" was related to the number of females that he could successfully breed within a relatively short period (Katz, 2008). To achieve success, males needed to have a high sex drive, motivation, and appropriate physical and reproductive capabilities (Emlen and Oring, 1977). Outcomes were determined competitively, with the advantage going to those possessing greater size, aggression, and weaponry in addition to sexual prowess. The result of this intense sexual selection among males was that most breeding males were of high sexual capability with relatively little variation occurring (Katz, 2008).

In contrast, by placing less emphasis on male competition, domestication has allowed males of lesser capabilities to be propagated. This was apparent in an early pig study (Sumption, 1961) in which unfettered natural selection led to increased male mating efficiency. The potential of artificial selection

to reduce male reproductive capabilities was evident in early Swedish observations in which selection of AI bulls on the basis of docility was accompanied by lowered sex drive, thus providing an example of pleiotropy, in which artificial selection for one trait (i.e., docility or tameness) was associated with unforeseen adverse consequences in another (i.e., male sex drive). Similarly, genetic correlations can occur between traits that may appear to be unrelated. For example, libido in beef bulls was negatively associated with production traits, such as average daily gain and final test weight (Ologun et al., 1981), as well as with breeding soundness assessments such as scrotal circumference and semen quality (Chenoweth et al., 1988).

Many common livestock management procedures are capable of systematically lowering male reproductive capability and performance. While such effects are unintended, they could compromise current management trends to decrease breeding season lengths and reduce male to female ratios; both of which place a premium on important male reproductive traits such as physical mobility, semen quality, and sex drive. Those management procedures that have been implicated in reducing male reproductive functionality include single-sire mating, inbreeding, artificial reproduction technologies (such as artificial insemination and in vitro fertilization), and the use of electro-ejaculation to obtain semen.

Injuries and pathologies

Breeding and management practices can also contribute to increased physical and pathological problems that adversely affect livestock reproductive behavior and performance (Ott, 1996). These include the effects of group composition, facility design, and feeding practices, all of which can cause undesirable competitive and antagonistic behaviors. Practices such as congregating animals within yards or pens (as in feedlots), or commingling animals from different backgrounds (as occurs with shows, sales, and postweaning transport), increase the opportunities for trauma and physical injury. For example, the likelihood of aggressive behavior in pigs is highest when they are introduced to each other for the first time and are of similar body weight (Rhydmer and Canario, 2014). Commingling can also facilitate the spread of pathogenic organisms, which cause disease with accompanying effects such as pyrexia, unease, depression, pain, and recumbency, all of which can suppress reproductive behaviors. Other examples of problems arising from modern management practices are discussed later.

One example is with rearing practices in which livestock males are managed in uni-sex groups (Price and Smith, 1984). This arrangement can continue far beyond puberty, such as with progeny testing of bulls. The unwanted consequences of this practice were illustrated in a study with young male goats (Price and Smith, 1984) in which subsequent heterosexual reproductive performance was compromised by homosexual attachments and

behaviors that developed over the rearing period. More direct physical problems can also occur with all-male rearing practices, such as those that commonly occur with young, purebred bulls being reared together in relatively small areas, such as pens or small pastures. Here, the combination of high-energy feeding and close social proximity provides both the urge and opportunity for bulls to mount each other, often with anal intromission. This scenario is conducive to lameness, penile damage, and accessory genital disease, all of which can compromise bull reproductive behavior, both in the short and long term

Lameness

Lameness deserves an important place in any discussion of reproduction, genetics, and behavior in domestic animals. Lameness appears to be increasing in prevalence in different livestock species, especially those under more intensive management. For example, it is regarded as the most serious dairy cattle welfare issue in a number of countries, a major cause of culling and euthanasia in sows, and a growing concern with beef cattle.

Lameness in livestock is a welfare concern because it causes pain and discomfort and compromises normal behavior. It is also of economic concern as it contributes to decreased production, lowered fertility, and reduced longevity of affected individuals in the herd or flock. The economic costs associated with lameness in dairy cattle include those associated with treatment and control methods, impaired reproduction, lowered milk yield, and decreased carcass value (Garbarino et al., 2004). Indeed, the treatment and control of lameness in itself consumes considerable labor and expense, spawning service industries such as foot trimming in cows and bulls.

Lameness can have a negative impact on reproductive behavior in both male and female livestock, especially when pain is involved. In females, it can suppress estrous and mounting behavior (Garbarino et al., 2004) and reduce their willingness to accept male mounting and service behavior, which is also susceptible to lameness. This last consideration is important with natural breeding, where male attributes such as good physical conformation, sexual motivation, and sperm production all contribute significantly to the desired outcomes. Considerable physical demands are often placed upon males as they often have to successfully service multiple females within a relatively short period of time and in a variety of environmental conditions and terrains. These demands take a toll as observed by Ellis et al. (2005) who recorded a 75% total injury rate, including a lameness rate of 63%, in yearling bulls used for natural breeding in Nebraska. Consideration of the capabilities of the male, however, should not be restricted to natural service. For elite males used as sperm donors for AI, those traits important for natural breeding success, such as good conformation, are often of lower priority than more marketable traits, thus allowing poor conformation to be

perpetuated. This risk was emphasized by the work of McNitt (1965) in which foot and leg conformation in bulls were strongly heritable.

A number of factors contribute to lameness, including conformation, nutrition, housing, and transport. Good conformation is an important consideration and one which is strongly influenced by genetics. For example, leg and feet defects were shown to be significantly heritable ($r = 0.59$) in inbred beef bulls in Colorado (McNitt, 1965), although estimates of heritability for claw lesions in dairy cattle were low, ranging from 0.03 to 0.16 across different models (Oberbauer et al., 2013). In turn, strong relationships occur between limb conformation and lameness (Van der Waaij et al., 2005; Franks and Grandin, 2015). Longevity of animals in herds and flocks, an indicator of animal management and welfare, is strongly influenced by conformation and lameness (Adamczyk et al., 2013). Genetics can also play an important, although unintended, role via pleiotropic effects such as in pig breeding where selection for rapid growth and carcass leanness is linked with increased foot and leg problems (Rauw et al., 1998). Poor conformation represents a major cause of culling in dairy cows (Zavadilová and Štípková, 2012) as well as in pigs (Le et al., 2016), where selection for good leg conformation in young pigs has been recommended for improved reproductive performance as well as sow longevity (Le et al., 2016. The Code of the European Forum of Animal Breeders (EFAB, 2013) emphasizes selecting against factors associated with leg and back problems in livestock.

Despite such considerations, the priority given to good locomotory conformation in livestock often ranks well below that given to production traits, some of which are themselves risk factors for lameness. As an example, poor foot and leg conformation is often encountered in resident semen donor bulls, both beef and diary, in the United States, European, and Australian bull studs, often with evidence of treatment for related pathological conditions. These bulls usually possess highly marketable production traits which assume precedence over other physical considerations. Thus it is perhaps not surprising that unsatisfactory physical conformation is often perpetuated by AI, particularly in purebred cattle. In addition, genetic defects that are more widely recognizable, such as spastic paresis and spastic syndrome, and that occur as genetic recessive traits, can be widely disseminated (Chenoweth, 1986). The observation that feet and lower limbs are often obscured in images in beef bull sale catalogs (Franks and Grandin, 2015) could suggest that inadequate attention is being given to good conformation and locomotion in elite beef bulls.

Conclusion

Ensuring that animal well-being and welfare are safeguarded in modern production systems requires a holistic approach in which production goals and related management procedures are aligned with current understanding in areas as diverse as applied animal behavior, physiology, ecology, environmental science,

genomics, and communication skills. Achieving this objective requires not only dedicated research, but also the appropriate acculturation and education of animal industry personnel so that the best knowledge and understanding are transformed into routine applications involving animal handling and management. Progress in all of these areas is essential for the sustainability of the livestock industries.

As domestication has had relatively little influence on innate animal behaviors, it could be assumed that management practices that reinforce and complement these would lead to optimal livestock management, production, behavior, and welfare (Spinka, 2006). Although this is good in theory, recent decades have severely tested the behavioral adaptability of domestic animals with increasing intensification of livestock systems in concert with strong selection for production traits. Overselection, inbreeding, inappropriate confinement systems, poor facility design, nutritional imbalances, and climate change have contributed to a litany of problems ranging from lowered fertility to abnormal behaviors and compromised animal welfare. Understanding and managing these side effects, while accommodating the environmental, social, and reproductive variations that accompany domestication (Jensen et al., 2008), should be an important research focus of animal science today. This emphasis is overdue, however, as "phenotypic and genetic responses to natural and human-created environmental variability are among the most neglected issues in domestication studies." (Larson et al., 2014).

Modern science and technology are undoubtedly capable of providing solutions, or appropriate alternatives, for many of the challenges that face livestock production today and tomorrow. The caveat is that this requires a motivated response as reflected in significant boosting of relevant research, education, and training. The rapidity with which these challenges are increasing would lend a sense of urgency for such a response.

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Chapter 6

Factors influencing the expression of behavior in the domestic dog

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ABSTRACT
Domestic dogs differ enormously in both their morphology and behavior. Numerous factors can influence the development and expression of canine behavior and, more generally, determine the success of the pet−owner relationship. This chapter considers the role of nature and nurture in shaping canine behavior. The influence of factors intrinsic to the animal is outlined, focusing on research that has explored the role of breed, sex, and cerebral lateralization in guiding canine behavior and cognitive functioning. The chapter goes on to consider the role of more extrinsic factors that can influence the development of dog behavior, discussing the contribution of early experience, source of acquisition, training techniques, and owner-related traits including personality and attachment style. The article points to the enormous amount of individual variation that exists between dogs and the myriad of factors that can work together to shape the behavior and functioning of the animal we see before us.

Introduction

The domestic dog, *Canis familiaris*, is one of more than 36 species of canid, a family belonging to the order Carnivora, and including, among others, the wolves, foxes, coyotes, and jackals. The canids are a widely distributed group of terrestrial predators that, with some exceptions, are relatively lithe and powerful in build and social in nature.

Many theories have been proposed over the years to explain the ancestry of the domestic dog (for reviews see Coppinger and Coppinger, 2001; Jensen, 2007; Miklosi, 2014). These range from the idea that the dog arose from a wild type of canid somewhat similar to today's Australian dingo, *Canis dingo*, or New Guinea singing dog, *Canis hallstromi*, to the notion that the dog has multiple ancestors, including, for example, the coyote, *Canis latrans* and golden jackal, *Canis aureus*. Genetic evidence now suggests that the wolf, and perhaps

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more specifically, the gray wolf, *Canis lupus*, is the closest living relative of the domestic dog (Vila et al., 1997; Wayne, 1993); whether or not the modern-day wolf is the true ancestor of the dog, however, is still under scrutiny.

There is also great debate as to where (e.g., Freedman et al., 2014; Thalmann et al., 2013; Wang et al., 2013), when (e.g., Germonpre et al., 2009; Vila et al., 1997), or even how many times (e.g., Botigue et al., 2017; Vila et al., 1997) the process of domestication actually took place. Up until recently, the fossil record was fairly consistent in suggesting that the dog was domesticated around the end of the last Ice Age, somewhere between 12 and 14,000 years ago. More recently, however, dog-like fossils excavated from two separate European sites have been dated to 33,000 years ago, although it is unclear whether these remains are ancestors of the modern-day dog or perhaps morphologically distinct wolves (Ovodov et al., 2011). Studies of molecular genetic markers have yielded contrasting results, although by and large serve to support the archeological evidence about the domestication of dogs. Molecular dating of 18 prehistoric canids, for example, suggests that domestication occurred somewhere between 18,800 and 32,100 years ago (Thalmann et al., 2013). Wang et al. (2013) similarly calculate that the split between wolves and dogs occurred about 32,000 years ago. A recent genetic analysis involving hundreds of breeds suggests that dogs may have been domesticated twice from two distinct wolf populations, once in Asia and once in Europe (Frantz et al., 2016). The debate surrounding the geographical and temporal origins of the domestic dog is likely to continue, but most of the evidence that is accumulating seems to favor multiple domestication events that took place between 14,000 and 33,000 years ago (see Bergstrom et al., 2020).

Domestication, and further artificial selection by humans, have given rise to a wide variety of dog breeds, all differing in both their physical appearance and behavior. While a dog's appearance is set in stone, its behavior is much more flexible and open to modification across the lifespan. A wide variety of factors can influence the behavior of any dog, although these can be loosely divided into variables that are intrinsic to the animal, that is, those that are more related to nature (e.g., breed, personality, sex, hemispheric specialization) and those that are extrinsic to the individual, that is, those that can be more attributed to nurture (e.g., early development, training, owner effects). This chapter considers the role of both nature- and nurture-related variables in shaping canine behavior.

Nature

The behavior of the domestic dog, like all animals, can be influenced by genetic predisposition and inherited traits. This section discusses the role of such variables in shaping canine behavior, focusing specifically on the influence of breed, sex, and cerebral lateralization.

Breed

Today, there are well over 400 breeds of domestic dogs, with newly recognized crossbreeds (e.g., labradoodles, schnoodles, cockapoos) gaining in popularity. Many of these breeds show enormous morphological diversity. Some (e.g., German Shepherd Dog, Japanese Akita, Siberian Husky) have retained their wolf-like appearance. Others (e.g., Poodles, Chihuahuas, Bichon Frise), however, have been highly neotenized and now bear little resemblance to their wild progenitor. The function of dogs in today's society is as diverse as their physical appearance. While most are kept exclusively for the purpose of companionship, many serve as working animals and are involved in, among other duties, the herding of sheep, guarding of property, detection of criminals, drugs, and explosives, and the provision of assistance for those with disabilities (for review see Coppinger and Coppinger, 2001).

To group dogs together, a variety of classification systems have been developed on the basis of the function for which individual breeds were originally selected. In the United Kingdom, the Kennel Club recognizes 221 breeds of dog, which it divides into seven groups (see Table 6.1). The American Kennel Club, likewise, recognizes seven groups of dog breeds but differs in terms of category names (in this case using the labels herding, hound, toy, nonsporting, sporting, terrier, working) and the breeds of dogs included in each cluster.

Studies exploring the evolutionary origins of behavioral traits tend to classify dogs using different systems yet again, in this case leaning toward

TABLE 6.1 An example of a commonly employed breed classification system (UK Kennel Club).

Group	Function	Example of breeds
Hounds	Hunting, tracking, and retrieving	Setters, Retrievers, Spaniels
Gundogs	Olfactory and sight hunting	Basenji, Beagles, Dachshunds
Working	Hunting, drafting, and guarding	Boxer, Doberman, Great Dane
Terriers	Catching vermin or other pests	Airedale, Cairn, Fox
Toy	Companionship	Bichon Frise, Chinese Crested, Affenpinscher
Utility	Working or guarding	Akita, Bulldog, Keeshond
Pastoral	Herding and guarding livestock	Australian Cattle Dog, Belgian Shepherd Dog, Border Collie

models that group dogs according to their genetic relatedness, for example, based on genomic analysis (vonHoldt et al., 2010). Dogs have therefore been classified into groups such as "Ancient" (e.g., Afghan Hound, Saluki), "Herding" (e.g., Border Collie, Welsh Corgi), "Hound" (e.g., Beagle, Borzoi), "Retriever-Mastiff" (e.g., Bernese Mountain Dog, Golden Retriever), or "Working" (German Shepherd dog, Toy Poodle) (see Konno et al., 2016).

The extreme variation that exists between dog breeds offers an exciting opportunity to study the genetic basis of canine temperament and the role of domestication and artificial selection on the evolution of behavior (Ostrander et al., 2000). Recent years have indeed witnessed increasing attention to research devoted to breed differences, both in terms of cognitive and behavioral traits, some of which is discussed below.

Breed differences in canine behavior

Breed differences in canine behavior have been studied using a variety of tools, including standardized tests and questionnaire-based surveys of dog owners, trainers, and behavioral experts (for reviews see Fratkin et al., 2013; Gartner, 2015; Rayment et al., 2016).

Standardized tests of canine behavior generally involve presenting dogs with a range of stimuli or situations (e.g., the sudden opening of an umbrella, the appearance of a person dressed as a "ghost") in a controlled manner and scoring the animal's response using a predefined set of criteria. Such tests are useful from an applied perspective, allowing for the assessment of behavioral traits that may help in the selection of dogs for assistance purposes, service work, or even facilitate the matching of animals with suitable owners (e.g., Alberghina et al., 2020; Asher et al., 2013; Bray et al., 2020).

Breed differences have been unearthed in studies using standardized tests of canine behavior. As early as the 1960s, Scott and Fuller (1965) studied the behavioral response of five dog breeds (Basenji, Beagle, Cocker Spaniel, Shetland Sheepdog, Wire-hair Fox Terrier) to a battery of tests from birth to over 1 year old. The research pointed to a strong effect of breed on the expression of numerous behavioral traits, including, among others, trainability, aggression, problem-solving ability, and emotional reactivity. A wide selection of standardized tests has been developed since Scott and Fuller's pioneering work, many of which also point to breed differences in behavior. For example, Svartberg's (2006) Dog Mentality Assessment has identified large differences between 31 breeds on behavioral traits of playfulness, curiosity/fearlessness, sociability, and aggressiveness, with findings pointing to an important role of artificial selection on breed-typical behavior. More recently, Kolm et al. (2020) analyzed observational data arising from human-directed play behavior in over 132 dog breeds, exploring for differences on the basis of breed function. Their data pointed to higher levels of human-centered play behavior in herding (e.g., Australian Shepherd, Border Collie,

Bouvier des Flandres) and sporting (e.g., American Water Spaniel, Golden Retriever, Labrador Retriever) breeds than nonsporting (e.g., Bichon Frise, Chow Chow, Tibetan Terrier) and toy (e.g., Toy Poodle, Pug, Yorkshire Terrier) breeds. This, coupled with selection for trainability and breed function, may have served an important role in the early domestication of dogs (see also Bradshaw et al., 2015; Horowitz and Hecht, 2016).

Given the logistical challenges (e.g., space demands, time restraints) in conducting behavioral tests, attention has turned to the use of surveys for assessing the same type of information. These tests are typically completed by the dog's owner or expert in the field (e.g., dog trainer, behaviorist, veterinarian) and require the individual to rate the animal on a number of behavioral traits (e.g., playfulness, fear of noises) using a Likert scale scoring system. The ratings arising are then combined and factor analyzed to create personality constructs, a methodology long used in the measurement of human personality. A number of canine personality questionnaires have been devised using this approach. These include, among others, the Monash Canine Personality Questionnaire, MCPQ (Ley et al., 2007), the Dog Personality Questionnaire, DPQ (Jones, 2009), the Positive and Negative Activation Scale, PANAS (Sheppard and Mills, 2002), the Vienna Dog Personality Test, VIDO-PET (Turcsan et al., 2018), and the Canine Behavioral Assessment and Research Questionnaire, C-BARQ (Hsu and Serpell, 2003). All tests differ somewhat in terms of the number, and nature, of traits employed to describe dog behavior, the number of factors identified, and the labels used to describe these, although most, if not all of these tests have been found to be reliable and valid tools for measuring canine personality. Moreover, consistency has been found between the use of more than one test, for example, the MCPQ and the DPQ (Posluns et al., 2017).

As with the standardized tests of behavior, breed differences have been reported using questionnaire-based methodologies. For example, in a study involving 455 Australian participants, working dogs and terriers were found to score more highly than other groups on the MCPQ-R subscale of "extraversion," while working dogs and gundogs rated more highly on "training focus" than other breed groups, notably toy dogs and hounds (Ley et al., 2009). Using the C-BARQ, Serpell and Hsu (2005) found highly significant differences between dog breeds for "trainability," with show-bred dogs having lower scores for this trait than field lines. Sporting and working dog breeds (e.g., Springer Spaniels, Labrador Retrievers, Poodles) were found to have higher scores for this factor, while dogs from other breed groups (e.g., hounds, terriers) had lower scores. Using the same assessment tool, Duffy et al. (2008) found that small dog breeds (e.g., Dachshunds, Jack Russell Terriers, Chihuahuas) have higher scores on many aggression factors, with other breeds (e.g., Pit Bull Terriers) only demonstrating higher scores in specific contexts, for example, dog-directed aggression.

One of the problems with research in this area to date has been the limited number of breeds and/or individual dogs recruited for participation.

Recently, however, MacLean et al. (2019) used the C-BARQ to examine variation in behavioral traits across 14,000 dogs from 101 breeds. The study revealed high levels of breed heritability for 14 behavioral traits, including behaviors such as trainability, stranger-directed aggression, attention seeking, and predatory chasing. Roughly 60%−70% of the variation in these traits across breeds could be attributed to genetics. Importantly, the authors discovered 131 sections of dog DNA tightly linked to these behaviors, with some found in genes involved in influencing behavior. While effects arising from environment, training, and other individual differences on a dog's behavior cannot be ignored (see later), the findings from this study emphasize the heritability of some complex traits and pave the way for further work aimed at exploring the genetic basis of canine behavior.

Breed differences in cognitive functioning

Not only has research identified breed differences in canine behavior, it also points to convincing differences in cognitive functioning. Some of the work in this area has focused its attention on problem-solving ability, with studies pointing to breed group differences in performance. For example, Hall et al. (2015) found that pugs, rather unexpectedly, outperformed German shepherd dogs on an olfactory discrimination task, while Marshall-Pescini et al. (2016) reported a superior performance of dogs in their working breed group than those in the retriever and herding (but not mastiff-like) groups on a manipulation task. This same study, and another investigation involving a detour task (Pongracz et al., 2005), however, failed to find any breed group differences, instead concluding that training experience was more likely to predict success outcome (see Section 3.2).

Breed differences in cognitive functioning appear to be more apparent on tasks that involve a communicative component with humans. Studies have shown that more ancient dogs (i.e., those that are more genetically similar to wolves), such as the dingo (Smith and Litchfield, 2009) and the New Guinea singing dog (Wobber et al., 2009), perform somewhere between wolves and modern-day dogs in their ability to use human communicative gestures, for example, pointing. Dogs may therefore have acquired cue-following abilities before the evolution of different breeds. That said, domestication has certainly played an important role in shaping this ability, with breeds developed to work more closely with people (e.g., shepherds, huskies) being more proficient at responding to gestural cues than dogs bred for nonworking purposes, for example, Basenji, Toy Poodle (Wobber et al., 2009). Even among the working breeds, differences in the ability to understand human gestures have been unearthed, with dogs bred to work in close cooperation with people and respond to visual signals (gun dogs, sheepdogs) being better in the use of pointing gestures than those bred to work more independently from humans, for example, hounds, earth dogs (Gacsi et al., 2009).

Breed differences in human-directed gaze have also been reported. For example, it has been shown that working breeds that rely strongly on visual cues (e.g., retrievers) gaze at humans for longer periods of time than herding breeds (German Shepherd Dogs) or dogs bred for companionship, for example, Poodles (Jakovcevic et al., 2010). More recently, Konno et al. (2016) found that, when required to undertake an unsolvable task, ancient breeds of dogs (e.g., Afghan Hounds, Akitas, Salukis, Shiba Inus, and Huskies) were less likely to establish eye contact with humans and gazed at people's faces for shorter periods of time, than a range of nonancient domestic dog breeds, including those bred for working purposes, for example, collies, retrievers, and shepherds. In a similar vein, it has been shown that dogs bred for hunting/herding (e.g., Australian shepherds, Border collies, retrievers), typically selected for their "cooperative" work with humans, looked at people's faces more during an unsolvable task than "primitive" (e.g., Akita, Alaskan Malamute, Samoyed, Siberian Husky) and "molossoid" (e.g., Boxer, Bull Terrier, Rottweiler) breeds (Passalacqua et al., 2011). Hory et al. (2013) provide further support for gazing behavior having a genetic basis, having found an association between human-directed gaze during an unsolvable task and polymorphisms in the canine DRD4 gene.

Taken together, the above studies support the idea that genetic changes arising from domestication and artificial selection for traits including cooperative working with people have contributed toward shaping dogs' personality and cognitive abilities, notably their sensitivity to communicative cues and human-directed gaze behavior (Persson et al., 2015).

Sex

Male and female dogs differ in a wide variety of biological domains, including brain size, anatomical structure, lateralization, and, of course, hormonal status. Given these fundamental differences in physiological and architectural underpinnings, it is perhaps not surprising that sex differences in canine behavior have been reported (for review see Scandurra et al., 2018).

Although the results arising from research on canine sex effects are by no means consistent, evidence points to differences in behavior that are rooted in dogs' biological and evolutionary inheritance. For example, compared to females, studies have reported that male dogs score more highly for traits of aggression and boldness (e.g., Starling et al., 2013; Svartberg and Forkman, 2002) and show more social contact in dog–human play (Asp et al., 2015; Strandberg et al., 2005). Females dogs, by contrast, have been shown to display higher levels of sociability (e.g., Foyer et al., 2013; Kubinyi et al., 2009), gaze-directed behavior toward humans, especially their owners (Duranton et al., 2016; Nagasawa et al., 2015; Wells and Hepper, 1999) and activity during certain types of training (Goddard and Beilharz, 1984). The types of sex effects reported in domestic dogs make sense if considered from

an evolutionary perspective. For example, the propensity for higher levels of aggression and/or boldness in males, compared to females, could be related to the fact that, in the wild, male animals often have to use aggression to defend territory or compete for access to females to achieve reproductive success.

Given the differences that exist in male and female dog behavior, it is perhaps not surprising to learn that the prevalence of behavior problems also differs between the sexes. Studies have reported a higher prevalence of behavior problems in male dogs compared to females (Dinwoodie et al., 2019; Wells and Hepper, 2000), with the former being more prone to aggression, particularly interdog aggression (Chung et al., 2016; Rooney and Bradshaw, 2004), training problems (Chung et al., 2016), inappropriate elimination (Landsberg, 1991), and mounting (Wells and Hepper, 2000). Female dogs, by contrast, are more prone to behavior problems including anxiety (Chung et al., 2016), phobias (Bamberger and Houpt, 2006), fearfulness (Didehban et al., 2020), and noise sensitivity (Storengen and Lingaas, 2015).

The effect of sex on canine behavior becomes more complex when one considers the impact of castration/sterilization (Reichler, 2009). Some studies point to what could be considered positive behavioral effects of this procedure. For example, neutered dogs of both sexes have been reported to be less bold (Starling et al., 2013), while castration of male dogs has been associated with reduced dog−dog aggression, marking, and roaming (Maarschalkerweerd et al., 1997; Neilson et al., 1997). Other studies, however, have reported results that could be considered more negative in nature (Chung et al., 2016; Dinwoodie et al., 2019). Thus research points to an increase in fearfulness and/or emotional reactivity following neutering (Balogh et al., 2018; Hakanen et al., 2020; Storengen and Lingaas, 2015; Zink et al., 2014). Other studies have found an increase in vocalizations and reactivity in sterilized female German shepherd dogs (Kim et al., 2005, 2006), and yet others have found spayed and neutered dogs to be more aggressive, excitable, and less trainable than intact animals (Farhoody and Zink, 2010). These effects, however, are by no means generalizable and, again, depend upon the sample group under investigation (e.g., breed, background), not to mention other variables including, for example, the timing of this event along the individual's lifespan, that is, pre- versus postpuberty (e.g., Starling et al., 2019). Indeed, it was recently concluded that the effect of neutering on dog behavior is inconsistent, and, in some cases contradictory (Ufer and Kaeberlein, 2019), so results from individual studies need to be interpreted with care.

Cerebral lateralization

The two hemispheres differ significantly in their functions, including emotional processing. The right hemisphere is generally considered to be responsible for the processing of fearful information, encouraging aggression or

withdrawal from novel stimuli and situations (e.g., Ahern and Schwartz, 1979). The left hemisphere, by contrast, largely inhibits fear, encouraging exploration and approach behavior (e.g., Silberman and Weingartner, 1986). In vertebrates, the hemispheres function contralaterally, with the operations of the left hemisphere expressed through the right side of the body and those of the right hemisphere expressed through the left-hand side of the body. Dominance, or more frequent use, of one hemisphere over the other predisposes individuals to behave in a certain way, opening the door for behavior to be used as an indicator of hemispheric lateralization and, indirectly, emotional processing. Much of this work indeed points to a link between side preferences and emotional functioning. Left-handed primates, for example, show greater reactivity, higher stress levels, and more fear responses than right-handed individuals (Braccini and Caine, 2009; Rogers, 2009, 2010). Gordon and Rogers (2015) found that left-handed marmosets (*Callithrix jacchus*) were more likely than right-handed animals to exhibit a negative cognitive bias, while nonlateralized chicks (*Gallus gallus domesticus*) have been shown to exhibit more distress calls in response to the sight of a predator than their more strongly lateralized counterparts (Dharmaretnam and Rogers, 2005).

The domestic dog has been studied extensively in relation to functional expressions of cerebral lateralization (for review see Siniscalchi et al., 2016). Much of this work has used paw preference as a tool for assessing motor bias (for review see Wells, 2021). Studies have been largely consistent in showing that dogs demonstrate a preferred paw use, although at the level of the individual, rather than the population. These paw preferences appear stable over time (Wells et al., 2018), but can be influenced by a wide variety of intrinsic and extrinsic factors, including, among others, temperament, mood, and demands of the task.

Some studies have pointed to sex differences in canine paw preferences, with females veering more toward right-paw use and males being more inclined to employ their left paw (Wells, 2003). Results are inconsistent in this respect, however, and a recent *meta*-analysis concluded that there was no evidence of a significant sex effect in directional laterality in dogs (Ocklenburg et al., 2019). Breed differences have not been found to be related to motor bias in dogs (McGreevy et al., 2010; Tomkins et al., 2010), although only a small selection of breeds have been studied so far in this respect (e.g., Whippets, Greyhounds, Boxers, Pugs). That said, shorter muzzle breeds have been reported to be quicker to reach test criterion on a "Kong ball task" (a challenge that requires an animal to stabilize a ball with its paw, see Fig. 6.1) than longer nosed breeds, which rely more heavily on their muzzle alone to retrieve the food (McGreevy et al., 2010).

An association between canine paw preference and emotional functioning has been reported. Compared to ambilateral animals, dogs showing a preferred paw use on the Kong ball test have been shown to display less noise sensitivity (Branson and Rogers, 2006), faster responses to catching a novel,



FIGURE 6.1 Dog undertaking the Kong ball test to assess paw preference.

moving object (perhaps reflecting a more confident personality, Batt et al., 2008), greater success on guide dog training outcomes (Batt et al., 2008; Tomkins et al., 2010), and weaker expressions of chronic stress in captivity (Demirbas et al., 2019). Increasing left-pawedness on the Kong ball test has also been associated with stress-related behaviors in a sample of rescue-sheltered dogs (Barnard et al., 2018) and a more "pessimistic" outlook on a cognitive bias challenge, with left-pawed animals being significantly slower to approach an empty food bowl placed in one of three ambiguous positions (Wells et al., 2017). A link been canine pawedness and immunity has also been discovered (Quaranta et al., 2004, 2006, 2008; Siniscalchi et al., 2010). Left-pawed dogs (as assessed by a sticky tape test, which requires the animal to remove a piece of adhesive tape from its nose) have been found to show a higher percentage of lymphocytes and a lower percentage of granulocytes than right-pawed or ambilateral animals (Quaranta et al., 2004). In a similar vein, left-pawed dogs immunized with the rabies vaccine have been shown to have lower titers of antibodies than their right-pawed or nonlateralized counterparts (Quaranta et al., 2006). Studies in this area point to evidence of brain asymmetry modulating immune responses in dogs.

Although subject to less empirical attention than paw preferences, other types of functional lateralization have also been reported in the domestic dog and appear to be linked to emotional processing and expressions of behavior. For example, asymmetric tail wagging has been reported, with both direction and amplitude of tail wagging related to the emotional valence of the visual

stimuli presented to the animals (Quaranta et al., 2007). Stimuli deemed to have a positive valence (e.g., the dog's owner) typically result in more tail wagging to the right-hand side, while those considered to be more negative in valence (e.g., an unfamiliar dog) tend to generate more tail wagging to the left-hand side. Acoustic stimuli also appear to be processed asymmetrically. Siniscalchi et al. (2008) found that dogs turned their heads more with their left ear leading in response to recorded sounds of a thunderstorm (an alarming stimulus), but turned their heads more with their right ear leading in response to the sounds of canine vocalizations, supporting the idea that the left hemisphere is involved in the analysis of familiar conspecific calls. Asymmetric olfactory responses have also been observed in dogs, although it must be borne in mind that the olfactory neural fibers are uncrossed and thus left nostril use reflects activation of the left (as opposed to the right) hemisphere. Studies have shown that dogs presented with arousing stimuli (e.g., adrenaline, veterinary sweat) sniff more with the right nostril (Siniscalchi et al., 2011), while left-nostril bias has been observed in dogs presented with odors collected from people during a fearful situation and physical stress (Siniscalchi et al., 2016). Interestingly, the same studies reported right nostril use in dogs presented with canine odor collected in a stressful situation, indicating that dogs use different sensory pathways to extract emotional information from conspecific and human chemosensory cues.

Taken together, the studies on lateralization highlight a strong link between functional asymmetry and emotional processing in dogs. The work emphasizes the role of cerebral asymmetry in shaping behavioral output and the merits of considering this issue in applied contexts.

Nurture

While a dog's behavior can be driven by biological underpinnings, the output that we witness in any one individual is a complex interaction of inherited and nongenetic factors. A wide variety of extrinsic variables can shape a dog's behavior, the role of which is discussed in the following section.

Source of acquisition and early experiences

Dogs can be acquired from numerous sources (e.g., pet stores, breeders, rescue kennels, puppy farms), an early experiential factor that can have a significant effect on an animal's behavioral development. Although results differ somewhat between investigations, by and large, studies point to the development of undesirable behavior patterns in dogs acquired from certain places. For example, Appleby et al. (2002) found the highest incidence of human-directed aggression in dogs that were raised in a kennel environment and in animals with no experience of living in an urban setting before 6 months old. McMillan et al. (2013) similarly reported that dogs purchased from pet stores

were more prone to stranger-directed aggression than animals acquired from noncommercial breeders. A recent review of dogs obtained from sources, including pet stores, commercial breeders, and puppy farms, highlighted the increased risk of fear-related behavior and aggression in commercially bred animals (McMillan, 2017). Breeders cannot necessarily be considered a uniform group, however, with outcome measures dependent upon a variety of variables. Indeed, Gray et al. (2016) found that the incidence of canine aggression and separation-related issues was higher in dogs obtained from "irresponsible," as opposed to "responsible," breeders (although it must be acknowledged that breeders were classified on the basis of their responses to a questionnaire). More recently, Wauthier and Williams (2018) explored the effects of puppy farming on dog behavior, using the mini-C-BARQ as a tool for assessing behavioral outcomes. Source of acquisition was found to have a negative influence on 11 out of the 14 C-BARQ subscales, including behavior and physical well-being. While these results largely confirmed those of earlier work in the area (McMillan et al., 2013), finding a higher prevalence of nervousness and aggression in dogs acquired from puppy farms, the study revealed no significant effect of source of acquisition on traits of "chasing," "energy," and "dog rivalry." These differences may be down to variations in approach taken to measure the outcome variables and/or differences in sampling. Of interest was the finding that certain owner-related behaviors, in particular walking and training, may go some way to mitigate the effects of poor early life experiences, although the authors urged caution in interpreting these results, with effect size being quite small.

The question remains as to why puppies acquired from kennel environments and similar institutional settings go on to develop problematic behaviors. A combination of genetic, prenatal, and developmental factors, which tend to be managed more poorly in commercial breeding establishments (HSVMA, 2013), are likely to play a role (McMillan, 2017). For example, certain undesirable behaviors, including aggression (Pérez-Guisado et al., 2006) and anxiety (Goddard and Beilharz, 1983) are known to be heritable. Bitches with these patterns of behavior may easily pass on a genetic predisposition to these problems to their offspring. Maternal stress (long known to be associated with adverse effects in offspring, e.g., Braastad, 1998) may exert an effect during the prenatal period, and/or go on to result in inappropriate care during the neonatal period (0−14 days), a factor known to have long-term consequences for the regulation of the hypothalamic-pituitary-adrenal axis and the development of physiological and behavioral responses to stress (e.g., Champagne et al., 2003). A potential lack of exposure to novel stimuli during the socialization period (∼3−12 weeks old) may exasperate the situation further. The importance of stimulus exposure during this timeframe has been recognized since Scott and Fuller's (1965) pioneering work; numerous studies now suggest that inappropriate socialization during the first 3 months of life can have a dramatic effect upon a dog's behavior, in some

cases leading to fear-induced behavioral problems (e.g., noise sensitivity, neophobia) that often persist into adulthood, and in many cases are resistant to modification. For example, large studies of Finnish pet dogs have found that animals that have been poorly socialized are more fearful and prone to noise sensitivity than those with more appropriate socialization experiences (Hakanen et al., 2020; Tiira and Lohi, 2015; Puurunen et al., 2020). Related to this, some puppies coming from kennel environments go on to be removed from the mother at too early an age (e.g., before 8 weeks old), during the all-important socialization period. Subsequent transportation, whether onwards to a pet shop, or further afield, may result in high stress levels and subsequent effects on the development of behavior.

Different breeds may be affected by these early life experiences in different ways, depending upon their sensitive periods of behavioral development. For example, German shepherd dogs tend to show stronger neophobia at 5 weeks old than Labrador retriever pups, which, by contrast, retain the ability to socialize for longer (Lord et al., 2016). There are obvious implications for this sort of breed difference in the early rearing of puppies, which may or may not be taken into account by commercial breeders and have long-term implications for behavioral development and, more generally, the success of the pet−owner relationship.

Training

Training can have a significant effect on canine behavior and influence both pets' and owners' quality of life and well-being. Dogs that have been trained have been shown to display fewer behavior problems (e.g., separation anxiety, dog-directed aggression, disobedience, destructiveness, Bennett and Rohlf, 2007; Clark and Boyer, 1993; Jagoe and Serpell, 1996) than untrained animals, while owners of trained dogs report higher levels of connection to their pets than owners who have not engaged in any training (Clark and Boyer, 1993).

Improvements in the pet−owner bond as a result of training are perhaps not surprising when one considers the effect that this type of activity has on a dog's social cognition. Animals that routinely undertake training activities with their owners, whether it be "agility," "dancing," or "search and rescue," have been shown to be more persistent in puzzle box tasks and look less toward their owners during problem-solving than untrained animals (Marshall-Pescini et al., 2008; Range et al., 2009). Interestingly, training has been reported to shape how dogs communicate with humans, with animals trained for agility being more inclined to look toward their owners during an unsolvable task and animals trained to undertake search and rescue being more inclined to combine looking at humans with barking and navigating more of their focus toward the experimenter than their owner (Marshall-Pescini et al., 2009).

The extent to which a dog gains benefits from training can be significantly influenced by the nature of the technique adopted to train the animal. Some people rely heavily on aversive methods (i.e., those that use punishment) as a teaching tool, while others focus more on positive reinforcement techniques involving verbal praise, touch, food treats, and/or the use of secondary conditioners, for example, clickers (Cooper et al., 2014; Haverbeke et al., 2008). Generally, studies have found that the use of aversive approaches to training has a negative effect on a dog's behavior and welfare. Arhant et al. (2010), for example, found a positive correlation between increased excitability and aggression and more frequent use of owner-induced punishment. More recently, de Castro et al. (2020) reported a negative effect of aversive training on canine welfare both within and outside the context of the training environment. Dogs exposed to punishment showed more pessimism on a cognitive bias task, more stress-related behaviors, and higher posttraining increments in cortisol levels than animals trained using positive reinforcement techniques. Interestingly, one study found no effect of aversive training (in this case the use of electric shock collars as a tool for refraining attacks on sheep) on canine fear or anxiety (Christiansen et al., 2001). This research, however, was based on owners' reports of their pets' behavior as opposed to more objective assessments of canine temperament (see earlier); the specific behavioral issue being addressed (i.e., predatory aggression) must also be borne in mind.

While training plays an important role in shaping canine behavior and welfare, the success of any training regime is bidirectional in nature, being dependent upon a variety of canine- and human-related factors. A dog's current age and age at the time of its acquisition, not to mention factors including temperament (see earlier), can all influence training success outcomes (Bennett and Rohlf, 2007; Hsu and Serpell, 2003; Kubinyi et al., 2009). Canine breed has also been shown to exert an effect. Marshall-Pescini et al. (2016) examined the performance of 128 dogs belonging to four breed groups (herding, mastiff-like, working, retrievers) on a manipulation and spatial detour task, comparing the performance within each group of highly trained dogs (i.e., animals that carried out a sport discipline at competition level or were employed as working animals for search and rescue, agility, tracking humans) versus nontrained dogs (i.e., animals that had no training or had been trained by their owners for general obedience, e.g., to sit, fetch a ball). Training background was found to have a significant effect on many of the outcome measures, with highly trained dogs having greater performance success overall and being faster to obtain a food reward on a spatial detour task than untrained animals. Breed group also exerted an effect on the manipulation task, with individuals in the working breed group, regardless of training background, being more likely to succeed than those in the retriever and herding groups. Animals in this latter group were also more inclined to look toward the person than dogs in the retriever and working (although not the mastiff-like) groups, a finding that contrasts with other work showing

breed differences in human gaze between herding and mastiff-type dogs (Passalacqua et al., 2011). Differences in both task demands and discrepancies in breed group classification may go some way to explaining the inconsistent results between these studies and the challenges presented by exploring breed-related effects in dogs more generally.

In addition to dog-related factors influencing training success outcomes, owner-related variables also play an important role. Indeed, Stevens et al. (2021) found that the cognitive ability of owners, perceived dog disobedience, and time spent training were the key factors shaping a dog's success on a Canine Good Citizen training program. Owners who invested the most time and energy in training their animals were the most likely to see successful training outcomes.

Owner traits

The role of parental (and notably maternal) personality in shaping the emotional, psychological, and behavioral development of children is well established (Conroy et al., 2012; Macedo et al., 2011). Given the similarities that exist between dog−owner and child−parent relationships, it is therefore not surprising to learn that canine behavior may be also influenced by owner-related traits.

Several studies have highlighted the role of owner personality in influencing various aspects of dog behavior, in particular the occurrence of behavior problems. Podberscek and Serpell (1997), for example, found that highly aggressive English cocker spaniels were more likely than animals with lower levels of aggression to have owners who were less stable, shy, undisciplined, and more prone to being tense. Turcsan et al. (2011), likewise, found parallels between owners' and dogs' personality profiles, with a positive correlation between both individuals' neuroticism scores. Interestingly, one study found little evidence of a relationship between owner personality and the occurrence of separation-related behavior in a sample of dogs, although pointed out that other factors besides those explored may account for the weakness of the relationship (Konok et al., 2015).

The question remains as to why owner personality should have an impact, or at the least, be correlated with, canine behavior. A number of explanations must be considered. At the outset of the dog−owner journey, owner personality may influence the type of dog acquired, with people perhaps leaning more toward breeds inclined to have similar personality traits (reputed or otherwise) to themselves. Wells and Hepper (2012), for example, reported higher scores for the trait of psychoticism in owners of dogs widely perceived to be "aggressive" (German Shepherd Dogs, Rottweilers) than in those generally considered to be "nonaggressive" (Labrador Retrievers, Golden Retrievers). It also seems to be the case that an owner's personality exerts an influence on the type of training they employ with their pet, which,

as discussed earlier (see Section 3.2.), can have a dramatic effect on a dog's behavior. Indeed, Kis et al. (2012) found that owners who were higher on traits of neuroticism and openness used more gestural and verbal commands when asking their pet to sit; owners higher on the trait of extraversion, however, tended to praise their dogs more.

What may be more important in influencing canine behavior than owner personality per se, is the match (or mismatch) between an owner's and dog's personality. Dube et al. (2020) found that the prevalence of canine behavior problems was higher in so-called "sensitive dogs," a personality trait that involves an enhanced perception of, and response to, sensory stimuli. This is perhaps not surprising given that sensory sensitivity in humans is associated with higher perceived stress levels (Benham, 2006); the findings from this study point to parallels in dogs of a sensitive nature. Of particular interest was the finding that canine behavior problems were more prevalent in situations where there was a mismatch between the owner's and pet's personality, especially if the owner scored lower on the sensitivity scale than the dog. Whether this mismatch in personality results in an inability of owners to empathize with their pets and perhaps a failure to provide for their needs, or whether owners who are very dissimilar to their dogs simply adopt a lifestyle that does not suit their pets is hard to determine. Either way, the findings from this study point to the role that individual differences can play in the success of the dog−owner relationship.

Related to the issue of owner personality is that of attachment, a reciprocal relationship that exists between an individual and a primary attachment figure. The bond that exists between a parent and their child has been known to play an important role in behavioral development since the work of Bowlby (1969) and Ainsworth (1969). Evidence now suggests that the type of attachment that develops between an owner and their dog can also have important implications for canine behavioral development. Some studies, many using an adapted form of Ainsworth's Strange Situation Procedure (ASSP), in which a dog is separated from, and then reunited with, their owner, point to an important association between an owner's attachment style and the animal's ability to cope with this stressful experience. For example, Siniscalchi et al. (2013) found that dogs of owners with a more "secure" attachment style behaved in a manner more similar to that of a securely attached child when separated from their owner. Similarly, Konok et al. (2015) reported that owners who scored higher on having an "insecure avoidance" attachment style were more likely to have dogs with separation anxiety. More recently, Rehn et al. (2017) assessed the attachment style of 51 Golden Retriever owners and observed the reactions of the owners and their dogs in response to a number of challenging situations involving the presentation of visual, auditory, and social stressors. Findings pointed to differences in how the dogs reacted to the various stressors on the basis of their owners' attachment styles and reported some parallels to findings arising

from parent−child dyads. For instance, dogs of "securely attached" owners were less likely to stand behind them during the approach of a visual stressor (a "ghost"), and oriented more to the auditory and visual stressors; the same dogs showed less attention to their owner during the visual stressors. By contrast, dogs of owners with a more "anxious" style of attachment were more oriented to their owner during a visual stressor, while dogs of more "avoidant" owners were less likely to stand close to their caregiver during the approach of a strange-looking person. Higher cortisol levels have also been found in dogs during the ASSP in animals whose owners were classified as having an "insecure-ambivalent" attachment toward their pet (Schoeberl et al., 2016).

Taken together, the studies in this area provide strong support for an important influence of owner-related variables and other extrinsic factors in shaping canine behavior and, importantly, an animal's ability to cope with challenging situations. Some of the work in this area points to interesting parallels between parent−child and owner−dog relationships. Further research is needed to explore these themes, both to determine whether there are shared underlying mechanisms and, from a more applied perspective, to establish the impact of these phenomena on the success of the dog−owner relationship.

Conclusions

The expression of behavior is complex. As discussed throughout this chapter, the behavior that we witness in any one dog is the result of an intricate interaction between nature-driven, and nurture-related, factors. A dog's behavior is very much shaped by both its biological underpinnings and individual environmental history, all steeped in the complex context of the dog−owner relationship. While any one dog might be genetically predisposed to behave in a certain way, whether by virtue of its breed, sex or personality, there is an enormous amount of variation between animals and a myriad of factors that will work together to shape the behavior of the animal we see in front of us.

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Chapter 7

From mind to genome: the effect of domestication on dog cognition and genetics

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ABSTRACT
Canine behavior has been studied for decades, but not until 1998 was it discovered that dogs have human-like cooperative communication skills that rival those of even our closest primate relatives. Ever since, canines have become subjects of increased research into the genetic underpinnings of these abilities. Here, we posit that domestication has been a driving force in the evolution of dog cognition. The latest technological advances have been instrumental in allowing us to have a better understanding of the impact of domestication on the canine genome, as well as the role that genetics play in dog behavior and cognition. Finally, we explore the ways this knowledge can be applied to better the lives of dogs and that of their human companions.

The interaction between nature and nurture has fascinated 8scientists for centuries. Central to this question is the origin of complex behavior and cognition, and within the past hundred years, dogs have become central to testing ideas about behavioral evolution. Here we review recent cognitive work and explore the genetic mechanisms through which dogs' socio-cognitive skills have evolved. With advances in cognitive and genomics research, there is strong evidence for cognitive evolution in dogs and the latest work is starting to pinpoint what genetic mechanisms might have been targeted during domestication.

One of the first large-scale behavioral genetics studies began in 1945 and focused on domestic dogs. Funded by the Rockefeller Foundation, the Jackson Laboratory was founded in Bar Harbor, Maine (Dewsbury, 2012;

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Freedman et al., 1961). After much deserved pushback on funding troubling eugenics research in the 1920s and 1930s, the Rockefeller Foundation sought to fund a more ethical "psychobiology" program using animal models. Part of their effort to investigate the genetics of mental illness and behavior included the largest study of dogs ever conducted. The "Bar Harbor Experiment," a 13-year study of dog behavior and genetics, involved breeding hundreds of dogs while controlling their rearing experiences. They tested each dog on a battery of behavioral and problem-solving tasks, with the ambitious goal to understand the role of heritability in shaping behavior and learning. This meant a focus not just on adult dogs, but on the development of behavior as well. Many of the measures were charted longitudinally across the different experimental groups to examine when different behaviors first emerged as well as their relative rates of development. The experiment focused on five dog breeds (beagles, basenjis, cocker spaniels, Shetland sheepdogs, and fox terriers). The five breeds showed marked differences in a variety of behaviors including barking, general trainability, and problem solving. Individuals from each breed were systematically crossed with other breeds to examine the relative contributions and interaction of genes and environment that produced the observed behavioral variability across the breeds (Dewsbury, 2012).

In these experiments, Scott and Fuller found evidence that these crosses affected the average and distribution of behavioral traits with respect to their purebred parents, providing some of the first evidence among dog breeds of heritability or variation attributable to genetic differences (Scott, 1964). Standardized practices for rearing and socialization, such as keeping litters together until 1 year old, were strictly followed. Twenty-six tests were performed with each dog, classified as one of four types of tests: performance, emotional reactivity, relationship, and physiological observation. Scott and Fuller calculated the percent of variance that breed differences could explain, and found that 27% of the variability in the measured behavioral traits could be explained by heredity. Just as importantly, Scott and Fuller also found evidence that the developmental environment largely influenced behavior as well. Puppies of different breeds socialized in a similar manner all showed similar responses to conspecifics and humans. Regardless of breed, early social exposure gave dogs confidence around others and the term "critical period of socialization" was coined. Ultimately, these experiments influenced our basic understanding of complex canine behavior across the fields of psychology, biology, and genetics.

Despite the Bar Harbor experiment and early comparison of dogs to humans, dogs did not become a conventional research subject for behavioral genetics until much later. Researchers often avoided dogs as subjects after initial failed experiments on dogs' "intelligence," such as when German scientist Bernhard Grzimek's sensational "counting" dogs were discovered to be really using human signals rather than mathematical abilities (Grzimek,

1941). Evolutionary researchers, who began focusing on how ecology shapes psychology, largely ignored dogs because they were viewed as products of artificial selection through interactions with humans (Miklosi, 2014). Dozens of other species, especially primates, received the attention of comparative psychologists, and thus canine cognition was largely unexplored until the 1990s (Hare and Woods, 2013).

We have learned more in the last 20 years about dog evolution, cognition, and behavior than we had learned in the previous century. Dogs today are a species of interest for biologists, psychologists, and, especially, geneticists, after the publication of the dog genome in 2005 (Lindblad-Toh et al.). As dogs are both ubiquitous in human societies and perform invaluable jobs for us such as service or detection work, the understanding of dog behavior is more important than ever. Advances in *both* genetics and cognition give us new tools to understand how genes affect canine behavior and cognition. Ultimately, work on canine cognition and more recent work on behavioral genetics advance our understanding of the canine mind and provide critical information to inform dog breeding, training, and selection for dogs in working capacities.

Cognitively remarkable

One evolutionary hypothesis suggests that humans show early emerging cooperative communicative skills that allow for the participation in culture and language acquisition. The earliest of these abilities, following pointing gestures and gaze direction are thought to be critical to normal human social development. So central are these abilities to being human that it was originally proposed that humans alone were able to follow cooperative-communicative gestures such as pointing or gaze. The hypothesis was in part based on the finding that nonhuman primates such as chimpanzees and capuchin monkeys were unable to spontaneously follow hand pointing gestures that indicated the location of hidden food (Anderson et al., 1995, 1996; Hare, 2011; Hare and Tomasello, 2004; Herrmann and Tomasello, 2006; MacLean et al., 2017; MacLean and Hare, 2015). Therefore, the understanding of communicative intent from another being was thought to be a uniquely human ability emerging between 9 and 15 months old (Carpenter et al., 1998; Tomasello and Call, 1997). However, in 1998, it was discovered that dogs are able to understand communicative gestures from human experimenters (Hare et al., 1998; Miklösi et al., 1998). Since then, dogs have been shown to reliably perform above chance on object choice tasks, in which a reward is hidden in one of two places and the experimenter indicates the correct hiding location with a gesture (e.g., pointing, eye gaze; see Fig. 7.1). Follow-up tasks have ruled out other possible explanations for this success including the use of auditory or olfactory cues (Bräuer et al., 2006; Rossano et al., 2014; Szetei et al., 2003). Overall, dogs' abilities in gesture



FIGURE 7.1 Pointing task. An experimenter hides food in one of two bowls and indicates the correct location with a pointing gesture.

comprehension appear to be highly flexible and more similar to those of humans than other great apes' (Hare and Tomasello, 2005; MacLean et al., 2017).

Dogs' social genius is not limited to following pointing gestures. Dogs are sensitive to humans' attentional states; they are more likely to obey a command when their owner is watching (Call et al., 2003; Schwab and Huber, 2006) and are more likely to steal treats when humans' backs are turned (Kaminski et al., 2013). In addition, dogs show signs of intentional communication. When an owner was either present or not present after a treat was hidden in an inaccessible location, dogs looked more frequently at both the treat location and the owner when the owner was in the room (Hare et al., 1998; Miklósi et al., 2000). Dogs are also successful at showing their owner which of four boxes a toy is hidden inside, using behaviors like gaze alternation between the owner and the box, and moving toward the box (Henschel et al., 2020).

The discovery of dogs' unusual skills in cooperative communication raises the question of the origin of these abilities. Did dogs inherit these abilities from wolves or were they shaped by domestication to understand humans in a new way?

The domestication hypothesis

Dogs have lived alongside humans for thousands of years; evidence of coburial with humans dates back to as early as 4300 BCE (Thomas et al.,

2021). Dogs descended from wolf ancestors, but there is much debate over both the location and nature. Some studies of ancient DNA support an East Asian origin (Brown et al., 2011; Savolainen et al., 2002), while other evidence suggests Middle Eastern or even European origin. (Ovodov et al., 2011; Pang et al., 2009; Vonholdt et al., 2010). Many argue that dog domestication is instead the result of multiple events and that dogs' diversity points to a multitude of genetic transfer episodes. Of equal interest to the physical place of origin, though, is the mechanism through which domestication took place and its effects on dog cognition.

The domestication hypothesis posits that the domestication of dogs as they evolved from their common ancestors with wolves resulted in cognitive evolution, especially in the social realm (Hare and Tomasello, 2005). It has been proposed that this evolution occurred in two main stages. In the first stage, individuals with a temperament that allowed them to approach human settlements and respond nonaggressively to humans were at a selective advantage. They could eat human refuse and waste around the outskirts of camps (Coppinger and Coppinger, 2001). This process began 15−25,000 years ago through interactions with foragers, not agriculturalists. In the second stage of domestication, dogs were intentionally or artificially selected by humans for a variety of traits. This second stage accelerated over the past 150 years resulting in dozens of genetically similar but phenotypically distinct breeds (Hare and Woods, 2021; Parker et al., 2010; Wobber et al., 2009). Each stage is posited to have commensurate cognitive change that resulted from the selection process. In the first stage, selection for attraction to humans allowed underlying variation in cooperative communicative abilities to emerge. This variation was previously hidden in regards to understanding human communication because it was overridden by the fear response: dogs' ancestors would flee before they ever had a chance to engage in cooperation with a human. Once selection altered dogs' temperaments and fear was replaced by an attraction to humans, they could apply their inherited social skills in new ways while interacting with humans (Hare and Tomasello, 2005). During the second stage of domestication, then, the variation in this ability that was now evident in proto-dogs could have been acted upon directly, through intentional selection for those individuals who best understood human's attempts to communicate, making them the best hunting partners, guardians, or companions. This direct selection for this new adaptation of preexisting skills would have led to further flexibility and adaptation in their cooperative communicative abilities.

Support for this hypothesis comes from several lines of evidence. First, dogs' cooperative communication abilities are variable and heritable—two characteristics that make it possible for them to have been acted upon by selection. Performance with human gestures in dogs varies independently from success in other cognitive tasks. Conventional laboratory and citizen science approaches have tested hundreds and even thousands of dogs on test

batteries that include dozens of cognitive tasks. Both types of data reveal the same pattern. Individual differences across different cooperative-communicative tasks are correlated with each other but not correlated with other tasks (Horschler et al., 2019; MacLean et al., 2017; Stewart et al., 2015). Additional examination of the citizen science data sets suggests breed differences in these skills are predicted by genetic similarity among breeds, are highly heritable, and are associated with genes expressed in the brain (Gnanadesikan et al., 2020a,b).

Second, the use of human gestures by dog puppies does not require intensive exposure to humans (Hare et al., 2002). Puppies as young as 6 weeks old are able to read multiple human gestures, with no significant difference between litter-reared and hand-reared puppies (Hare et al., 2002; Riedel et al., 2008; Virányi et al., 2008). A large study of over 300 litter-reared assistance dog puppies showed that they are able to understand these gestures without repetition, with 70% of puppies succeeding in the very first trial (Bray et al., 2021a). Even free ranging dog puppies, who are not directly cared for by humans at all, show this ability (Bhattacharjee et al., 2020). While dogs can become more skillful at reading human gestures with age and training (Bray et al., 2021b; Watowich et al., 2020), dogs seem prepared to cooperate and communicate with humans, and relatively little exposure or experience with humans is needed for them to express this ability.

Domesticated foxes

The third line of evidence lending support to the domestication hypothesis is that experiments in other species, in which experimenters artificially select for the same temperamental traits as are proposed to have been favored during the first stage of domestication, result in similar cognitive changes as we see in dogs. The most well-known series of these experiments took place over 60 years, with farmed foxes in Russia. Beginning in 1959, a group of foxes were bred selectively, solely based on their response toward humans, allowing about 20% of the least fearful and aggressive in each generation to interbreed for about 40 generations. The resulting foxes had noticeably different physical characteristics, such as floppy ears and splotchy coats, despite only being selected for temperament (Trut, 1999; but see Lord et al., 2020). Even more interestingly, their pattern of social development changed: they became socially gregarious toward humans earlier in development than the control foxes, and retained this temperament into adulthood while the control foxes only had a brief window of this prosocial behavior toward humans as juveniles. Experimental fox kits also used human gestures at the level of dog puppies, while the age-matched control foxes (bred irrespective of their response to humans) did not. They show more skill with two different communicative tasks even though the control foxes were raised with intensive exposure to humans and outperformed the experimental foxes on a nonsocial

task. This work led to the proposal that a similar process occurred during dog domestication and led to the expression of dogs' unusual social skills (Hare and Tomasello, 2005).

In addition, other discoveries suggest evolved biological mechanisms at play. Analysis of this same group of foxes revealed decreased HPA axis activity in the tame line, and pointed to hypothalamic peptides as a mechanism through which the foxes, and dogs, evolved reduced aggression (Saetre et al., 2004). In addition, it was discovered that, similar to humans, dogs may have an oxytocin loop, in which mutual gaze between dogs and humans increases urinary oxytocin, a hormone involved in regulating social bonding (Nagasawa et al., 2015). Both of these mechanisms are evidence of hormonal level changes as a result of domestication.

Comparing wolves and dogs

Critically, the domestication hypothesis predicts that the early emerging use of human gestures will only be present in dogs and not wolves. Until recently, there was limited evidence available to support this prediction. While some adult wolves succeed using human social gestures, they seem to require intensive exposure to humans or explicit training (Gácsi et al., 2009; Hare et al., 2002; Lampe et al., 2017; Udell et al., 2012; Virányi et al., 2008). The critical test is to explore differences between dogs and wolves when they are young puppies. Virányi et al. (2008) raised a group of wolf puppies with similar exposure to humans as a comparison group of dog puppies, but the wolves ($N = 8$), at 4 months old, did not spontaneously read human pointing gestures when first tested.

Replicating and extending these findings, in a more recent study, a large sample of wolf puppies ($N = 37$) were raised with intensive exposure to humans within the first weeks of life. The majority of these wolf pups were with a human caretaker 24 h a day—even sleeping together in their beds at night. They were compared to a similar-sized sample of assistance dog puppies who were litter-reared with minimal human exposure. The results showed a difference in precisely the areas that would be predicted by the domestication hypothesis. First, in their temperament, dog puppies showed more attraction toward humans, being more willing to approach both familiar and unfamiliar people than wolf puppies. Second, in their performance on two cooperative communication tasks, dog puppies better comprehend humans' cooperative gestures, and they also make more eye contact with people when attempting to solve a difficult problem (Salomons et al., 2021; see Fig. 7.2). On nonsocial cognitive tasks, there was no difference between the dogs' and wolves' performance (see also Brucks et al., 2019). In the only other previous comparison of such young puppies, Gácsi et al. (2009) did not report a difference in gesture comprehension between wolf and dog puppies. However, the wolf and dog puppies were equally unskilled. The small

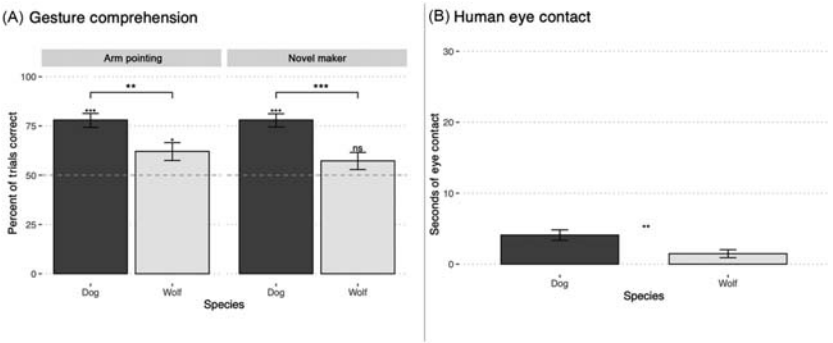


FIGURE 7.2 Tasks measuring cooperative communication with humans in dogs and wolves. (A) Estimated probability (± SE) of subjects choosing the location indicated by the experimenter's gesture in the gesture comprehension tasks, with a dashed line at the level of chance (50%). (B) Mean total number of seconds (± SEM) subjects spent looking at a human face across four 30 s trials in the human eye contact task.

sample of puppies ($n = 6$) from both species were just marginally above chance using a human pointing gesture. There was likely too little power to detect the species difference observed in other studies. Currently, there is no evidence for the spontaneous use of human gestures in wolf puppies like that seen across a range of studies in dogs and dog puppies (Salomons et al., 2021). In contrast to dog puppies, wolves appear to need intensive exposure to humans if they are to eventually learn to read human gestures (Virányi et al., 2008). Furthermore, the ability to read human social gestures has been shown in dingoes and New Guinea singing dogs, who have not undergone the intense selection pressures of the pet dog (Smith and Litchfield, 2010; Wobber and Hare, 2009).

What genetic mechanism did evolution target?

Evolved differences in the cooperative-communicative skills between dogs and wolves, which are early emerging and do not depend on intensive experience, suggest that these skills are biologically prepared (Cummins and Cummins, 1999). The question then becomes: what genetic mechanisms may have been altered during dog evolution that led to the evolved phenotypic differences we observe? The first research into genetic differences between dogs and wolves supports the hypothesis that the selection pressures of domestication altered dogs' cognitive development. Studies of domestication have revealed that compared to domestic dogs, wild canids have a distinct signature of expression of certain genes in brain regions such as the hypothalamus and prefrontal cortex. Divergence of gene expression in these regions, which are linked to exploratory behavior and emotional responses in these animals, is likely targets during such a selection on behavioral traits

(Li et al., 2013; Saetre et al., 2004). Whole-genome sequencing has also revealed key differences between dogs and wolves that are specifically related to brain function and development (Pendleton et al., 2018; Wilkins et al., 2014). In a list of candidate genes thought to be under selection in dogs, many are involved in neurological processes. This includes genes modulating the serotonergic system which could be partly responsible for the reduced aggression toward humans implicated in domestication, as well as allowing dogs to adapt to crowded living environments (Wang et al., 2013). Additionally, the neural crest hypothesis supports these results, postulating that neural crest cells are responsible for common traits seen in domesticated animals, such as a prolonged juvenile period, furry coats, tameness, reduced tooth size, and floppy ears (Wilkins et al., 2014). Based on this hypothesis, seemingly unrelated traits are all closely linked via pluripotent stem cells. Thus this unified, mechanistic hypothesis explains how domestication, via genetic variants that influence neural crest cell migration or numbers, causes unselected byproducts seen in dogs today in addition to more obvious behavioral traits like reduced fear. More recent whole-genome comparisons of wolves and village dogs support the neural crest theory, finding that genes involved in early embryogenesis differentiate dogs and wolves and that these signatures resulted from domestication rather than recent breed formation (Pendleton et al., 2018). Future research in this area may shed more light on the exact impact of these genetic and epigenetic changes, and potentially reveal the specific genetic pathways related to the early-emerging cooperative communication skills seen in dogs.

Different types of cognition?

In early research on dogs' communicative skills, traits were often investigated with the goal of understanding if a group of 10–30 dogs could solve a problem. This type of evidence was then used to infer if the entire species or a breed possessed different forms of cognition. More recently, hundreds or even thousands of dogs have participated in tests measuring their behavior and cognition. These larger samples allow for the measurement of variation both within and between individuals, as well as between groups.

Stewart et al. (2015) is the first demonstration of different types of cognitive domains in dogs (and maybe animals, see Herrmann et al., 2010). Examining individual differences revealed cognitive performance across domains varies independently. Doing well in one cognitive domain did not predict strong performance in another domain (and vice versa). Dogs seem to have at least 4–6 domains of cognitive ability that include memory, communication, inhibition, and social attention. The individual structure of these domains—especially the intercorrelation between social tasks and nonsocial tasks in dogs—is more similar to that observed in human children than in chimpanzees (MacLean & Hare, 2018). Each cognitive ability becomes

another trait that can be examined to understand the role that genetics play in creating individual differences.

The opportunity to investigate the genetics of breed differences

Dogs have a wide variety of roles in human society that vary on a continuum, with some dogs functioning as human family members and others in a strictly working capacity (Miklosi, 2014). In addition, specific dog breeds are often thought to be well suited (or not well suited) to specific roles along this continuum. The idea that certain breeds have been selected for different types of work has suggested there may be a genetic basis for these differences. Ethical concerns rule out some precise methods used in mice that determine gene functionality, such as genetic engineering to transfect novel genes into animals or knock-in/out gene functions (National Research Council US, 2002). Instead, research on dogs has focused on finding statistical relationships across different dog breeds. This method, though, may introduce a host of problems, as well as provide an amazing opportunity to understand the phenotypic variation typified by differences in dog breeds.

It is important to define what is meant when we refer to a dog population as a breed. In general, after splitting from their common ancestor with wolves thousands of years ago, populations of dogs were genetically isolated, inbred, and crossed to "fix" specific phenotypic traits (Boyko et al., 2010; Lindblad-Toh et al., 2005). Although different varieties of dogs existed in different parts of the world, it was not until the 1800s that intentional, controlled breeding based on both outward appearance and desirable abilities (e.g., herding or hunting) began. Breeding clubs, such as the American Kennel Club (AKC) in the United States, were created as official gatekeepers of breed certification, requiring registration of dogs to further limit the genetic diversity (American Kennel Club, 2006). The result was the creation of relatively homogeneous dog populations (Parker, 2012). Today, approximately 400 breeds exist, 197 of which are currently recognized by the American Kennel Club.

Before discussing the usefulness of breed analyses in canine behavioral science, it should be noted that breeds are not a perfect discriminatory factor for behavioral differences. For example, one early study assessed 56 different breeds on 13 different traits (including excitability and alert barking), with the goal of creating "behavioral profiles." The results showed that some traits showed greater between-breed differences than others (in other words, not all behavioral traits of interest were attributable to breed) (Hart and Miller, 1985). In addition, Pongrácz et al. (2005) found that breed was not responsible for differences in ability to learn from another human how to complete a detour task. In addition, Helton and Helton (2010) found that the physical size of a dog, not a dog breed, was the best predictor of performance on a

pointing gesture task. In addition, absolute brain size was a better predictor than breed specifically for a measure of a dog's propensity to steal food, as larger dogs outperformed smaller ones on memory and self-control tasks (Horschler et al., 2019). These differences do leave a large role for environmental influence as a potential explanation. For example, the living environments of small dogs may, on average, meaningfully differ from those of larger dogs. Small dogs may receive less or a different type of training.

Despite the potentially subjective origin of dog breed classification and these mixed behavioral findings, many trait differences have been identified between breed groups in their genes, brains, and behavior. The publication of the dog genome in 2005 revolutionized the field of dog genetics, making it possible to identify single nucleotide polymorphisms across breeds and identify genes responsible for disease and behavior (Lindblad-Toh et al., 2005). Thanks to the now widely available genome sequencing of dog breeds, breed designations have been used as proxies for genetically distinct populations. In an analysis of molecular variance, it was estimated that breed differences account for 27% of genetic variance in dogs (Parker et al., 2004). Single nucleotide polymorphism (SNP) analyses of breeds validate large amounts of genetic variance between breeds due to inbreeding and selection (vonHoldt et al., 2010), and microsatellite markers that discriminate between breeds can be used to predict breed classification with 99% accuracy. By and large, this phenotypic diversity is also a huge asset in the exploration of canine genetics. The results of very strong selection for specific behavioral and physical traits, breeds are a selection experiment simply waiting to be analyzed.

To this end, the Canine Behavioral Assessment Research Questionnaire (C-BARQ), one of the most widely used survey measures of canine behavior is often used in conjunction with SNP data to investigate specific genes' influence on behavioral outcomes. The CBARQ has found reliable, independent breed averages on 14 metrics, ranging from dog-directed aggression to attachment and attention seeking (Hsu and Serpell, 2003). Further work (MacLean et al., 2019) has confirmed that C-BARQ metrics are in fact highly heritable.

The population structure of dogs and breed-specific behavioral profiles allow for ample opportunity to explore how breed groups, after years of intense artificial selection have affected underlying neuroanatomical development in dogs. With advances in neuroimaging techniques, we are now able to investigate neural traits associated with breed differences. Not only are there neuroanatomical differences between dogs of different breeds, as is visible in MRI scans, but regional volumetric variation is distributed nonrandomly throughout the brain (Hecht et al., 2019). After controlling for brain size, total body size, and skull shape, six regional subnetworks were found to maximally explain this variation between breeds. Further analysis of these dogs used the C-BARQ to explore how well these neural differences could

explain breed differences in temperament and found that several behavioral categories (e.g., excitability, stranger-directed aggression, touch sensitivity) were associated with the same gray matter networks as well as relative expansion of specific brain regions over others (Hecht et al., 2021).

Evidence supports the idea that dogs were bred for specific temperament traits like those measured by the C-BARQ. It also raises the possibility that artificial selection shaped specific skills and behavioral specializations in different dog breeds. For example, some breeds may be more skilled at understanding human communicative gestures when solving problems. While almost all breeds have shown ability in following human gestures above-chance level, "working" dog breeds such as shepherds and huskies performed better in an initial comparison to nonworking breeds. This suggests that two waves of selection may have acted on dogs' social skills. The first was driven by natural selection during the initial stages of domestication and gave the species unusual skill at reading humans. The second wave is the result of intentional selection by humans when breeding dogs for roles in which cooperative communication is important for their work (Wobber et al., 2009).

It is also possible that the type of work dogs are bred for may influence their responsiveness to human gestures. Udell et al. (2014) found that breeds that have been bred for jobs that require enhanced predatory response, specifically the "eye > stalk > chase" sequence, outperformed a breed that had been bred for inhibited predatory response. The biological predisposition to orient toward and chase movement may thus further enhance the response to a pointing gesture.

Breed differences also exist in gaze following and eye contact behaviors. Retrievers have been found to make more eye contact with humans than poodles and German shepherds when food is in sight but out of the dog's reach (Jakovcevic et al., 2010). Similarly, Passalacqua et al. (2011) found that as adults, "hunting/herding" breeds made significantly more eye contact with humans in an unobtainable food context than did "primitive" or "molossoid" breeds, as grouped by a genome analysis. Breed comparisons of brain structure volume further support these behavioral differences; MRI scans of dogs of different breeds reveal distinct neural correlates of behavioral specializations like sight hunting, guarding, and companionship (Hecht et al., 2019). Importantly, nonrandom variations in regional subnetworks are not simply the result of total brain size, total body size, or skull shape. Phylogenetic analyses indicated that these changes were the result of recent selection. Finally, these differences appear to emerge relatively early in puppies' development—while not present at 2 months old, by 4.5 months old the "hunting/herding" dogs made significantly more eye contact than dogs of the other two breed groups.

But what about identifying specific genes that explain physical and cognitive diversity in dogs? A small number of loci explain large amounts of

physical variation in dogs (Boyko et al., 2010). Using simultaneous whole-genome association mapping, loci were identified that are associated with variations in physical traits such as body size, skull shape, and curl and length of hair (Ostrander, 2021). This technique has great potential to be used for learning more about the loci and genes associated with complex behavioral traits as well.

We are beginning to discover the genetic variations associated with these breed differences. While breed differences have not been narrowed down to a few loci (these complex traits are likely highly polygenic), progress is being made. Plassais et al. (2019) analyzed whole-genome sequences of 144 different AKC-recognized dog breeds, 54 wild canids, and 100 village dogs using a technique called GWAS (Genome Wide Association Study; a technique allowing for searches across whole genomes for genes or loci that have a high correlation with individual differences in a phenotypic trait of interest). They found genes strongly associated with physical traits and were also able to identify a specific locus associated with the variation in aggression between breeds. MacLean et al. in (2019) took this further, by obtaining measured data from professional behavior assessments of various breeds, and found 131 single-nucleotide polymorphisms from genetic databases that associated with 14 different behavioral traits that differed between breeds including trainability, excitability, chasing, aggression, and fear. Similarly, a GWAS examined performance on cognitive tasks from a large citizen science dataset and found 188 genes associated with breed differences in inhibitory control, communication, memory, and physical reasoning (Gnanadesikan et al., 2020a).

It is important to acknowledge that for any GWAS, an association between a locus or gene and a particular trait does not necessarily imply causation. To truly test the causative relationship, gene manipulation studies would be necessary, which would be unethical in most research with dogs. However, emerging evidence indicates that a number of the identified genes associated with these behavioral and cognitive traits may be functionally relevant. In both the MacLean et al. (2019) and Gnanadesikan et al. (2020a) study, a significant number of the identified genes associated with behavioral and cognitive differences are expressed in brain and nervous system tissue. These included genes like the EML1 gene that is known to drive neurogenesis and is associated with breed averages for performance on inhibitory control tasks. Future research can further test whether individual measures on behavioral and cognitive tasks replicate these breed-level findings. Collecting a large enough dataset to conduct a GWAS in this way is quite an imposing task, but citizen science may be a way forward. For example, the Dog Aging Project (Kaeberlein et al., 2016) is currently attempting to do something similar to find genes associated with common diseases in dog aging, by asking pet dog owners to send in DNA samples and report their

dog's health throughout their lifetime. In the meantime, using breeds to link these traits with representative genomes is an incredibly useful tool.

Heritable forms of cognition?

The domestication hypothesis posits that dogs' remarkable social cognition evolved as a result of selection, first for temperament (friendly toward humans), and then perhaps directly for cooperative-communicative skills. We know that for a trait to evolve via selection, it must, of course, be heritable. We have so far reviewed evidence that there are genetic (i.e., heritable) components to temperament, but what about cognitive traits? Researchers have recently discovered that many social cognitive skills in dogs are highly heritable (Bray et al., 2021a; Gnanadesikan et al., 2020a,b). Bray et al. (2021a) tested 375 8-week-old assistance dog puppies on a battery of cognitive and temperament tasks. Each puppy has detailed pedigree records going back generations, so the researchers were then able to analyze their genetic relatedness in correlation with their performance on these tasks. Two tasks stood out as being highly heritable with relatedness predicting performance: attention to human faces, and the ability to follow a human's pointing gesture. Performance on both tasks revealed that over 40% of the individual variation was attributable to heritable factors.

Applications

Research on how genetics contribute to dog behavior and cognition has potential applications. This is especially the case for working dogs that play important roles in society as assistants to people with disabilities, scent detectors, livestock protectors, providers of emotional support, and much more. In fact, demand for dogs who can succeed in these varied roles is only growing (Otto et al., 2019), and advances in canine behavioral and cognitive science can help match this demand through enhanced breeding, selection, and training of working dogs (Hare & Ferrans, 2021).

As we have shown, many behavioral and cognitive traits in dogs are heritable and have been successfully shown to be associated with specific genetic factors. Recent studies have found that a number of these traits are useful in predicting success in working dog roles. MacLean and Hare (2018) found that propensity to make eye contact when faced with either an unsolvable task or an interruption during a joint activity predicts training success for assistance dogs. They also found the most successful explosive detection dogs were those who scored highest in their responsiveness to human communication. Furthermore, these traits appear to be stable over an individual assistance dog's development from puppyhood to adulthood (2 years old), further enhancing their usefulness as predictive tools (Bray et al., 2021b). For scent detection dogs, we see a similar pattern. Differences in search-

related behaviors and reactions to novel stimuli, which help predict which dogs will succeed at training, emerged as early as 3 months (Lazarowski et al., 2018). Dogs who made more eye contact with humans during an unsolvable task at 11 months old were more likely to be successfully placed as detection dogs at 12 months (Lazarowski et al., 2019).

Behavioral and cognitive traits can be useful tools for predicting success in many different working dog roles. We now have evidence that genetics play a role in the development of these traits. The challenge that remains is applying this knowledge practically for use with a variety of working dog types. Across working dog organizations, methods for evaluating behavior vary greatly and are often subjective and qualitative (for a comprehensive review of assessments and their relation to working-dog outcomes, see Bray et al., 2021b). As Lazarowski et al. point out in their 2019 paper on the future of detector dog research, "focusing on the identification of the biological bases of such underlying characteristics presents a promising approach identifying underlying biological mechanisms will allow for the quantification of traditionally subjective measures and for assessments of the effects of biological factors." This knowledge can be utilized to enhance both breeding and selection programs for working dogs. For example, we now know that there is high heritability for traits such as fetching tendency or fear of loud noises and that over 40% of the variance in performance on measures that are known to be predictors of assistance dog success is explained by genetics (Bray et al., 2021b; Ilska et al., 2017; Wilsson & Sundgren, 1997). This means that these traits can be selected on and increased or decreased through breeding, potentially increasing the proportion of successful dogs in future litters. The same could be done for other traits that are important to working-dog performance that is discovered to have strong genetic contributions. Additionally, as more specific genetic markers are identified that underlie these useful traits, genetic screening could be implemented as part of the selection process for which puppies to invest in further training. For example, specific olfactory receptor genes have been identified that are present in the best scent detection dogs (Sacharczuk et al., 2019), the MYH9 gene has been highly associated with heat tolerance in sled dogs (Huson et al., 2011), and a variable number tandem repeat in a particular gene associated with neurotransmitters is associated with impulsivity and predictive of success in military dog training (Eo & Kim, 2013). Theoretically, puppies could potentially be screened for genes that are associated with useful traits for their jobs, and organizations could begin to devote their time and financial resources earlier toward the puppies with the highest likelihood for success, while adopting out those better suited as pets before investing in their professional training. There is still much more research to be done before genetic screening can be reliably utilized as a tool for working dog selection, but significant progress is occurring (Bray et al., 2021a).

Conclusion

Since the early days of the Bar Harbor experiment, great strides have been made in exploring how genetics contribute to dog behavior and cognition. We have learned that of the various cognitive domains, dogs stand out in their remarkable social skills: their cooperative-communicative abilities in particular are more human-like than those of even our closest phylogenetic relatives. The domestication hypothesis suggests that the selection for a friendly temperament toward humans in the early stages of dog domestication brought along with it a suite of changes, including an ability to express their cooperative communicative skills with humans, which had been inhibited by fear in their ancestors. The evidence from young puppies, a fox domestication experiment, and comparison with wolves all support this hypothesis and lead us to explore more precisely how genetics are contributing to dogs' cognition. We have been able to use GWAS to further investigate particular genes that are associated with variation in cognitive traits among breed groups. Within breeds, we have been able to assess that some of these traits are indeed highly heritable. In the future, if a large-scale cognitive testing battery were conducted with enough individuals that a GWAS could be done to directly correlate particular genes with cognitive performance in the same individuals. This would provide a more finely tuned list of potential genes to investigate when looking for causal mechanisms to relate genes to cognitive abilities. This knowledge can enable us to provide the best care, training, and enrichment for our dogs. Given that they are not only our best friends but also perform such crucial jobs, such knowledge can only improve the lives of both our species.

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Chapter 8

Genetics of domesticated behavior in dogs and foxes

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ABSTRACT
Differences in the behavior of the domestic dog (*Canis lupus familiaris*) and its progenitor species, the gray wolf (*Canis lupus*), are well recognized but the mechanisms of the wolf to dog transformation remain an area of scientific debate. A view of dog domestication that is centered on genetic selection for behavioral traits receives support from the famous Russian farm-fox experiment that began in the 1950s. Selection of foxes (*Vulpes vulpes*), separately, for tame and for aggressive behavior, has yielded two strains with markedly different, genetically determined, behavioral phenotypes. Tame-strain foxes communicate with humans in a positive manner and are eager to establish human contact. Conversely, foxes from the aggressive strain are aggressive to humans and difficult to handle. Although selected solely for behavior, changes in physiology, morphology, and appearance with significant parallels to characteristics of the domestic dog were observed in the selected strains. The genetic analysis of the fox populations identified several genomic regions that are homologous to the regions in the dog genome that differentiate dogs from wolves. Although the genetic regulation of domesticated behavior is far from being completely understood enormous progress has been made in this field. This chapter reviews studies of behavior and genetics in dogs and foxes and highlights the role of selection for behavior in ancient and modern dog formation.

This chapter offers an overview of recent studies in behavior and behavioral genetics of dogs, wolves, and foxes with the aim of providing insight into the complex structure of domesticated behavior in canids and the role of selection for behavior in dog domestication.

* Retired

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All domesticated animals have behavioral features distinguishing them from their wild counterparts. These characteristics can differ among species but in general, domesticated animals demonstrate reduced aggressiveness and lower sensitivity to environmental changes (Price, 2002). This complex suite of behaviors is often described as tameness. In contrast to wild species, which can be tamed if hand-raised from an early age, domesticated species require very little human socialization. Tame behavior of domesticated species has a strong genetic component, and it is passed from generation to generation while tame behavior of tamable species is a learned behavior and can be obtained only through extensive socialization of every new generation (Coppinger and Coppinger, 2001; Zeder, 2006). The timeline of behavioral changes that took place during the wolf to dog transformation is unknown but it has been hypothesized that the development of the abilities to coexist with humans was an important step in dog domestication that preceded the development of dogs' social human-directed skills (Ballard and Wilson, 2019). Furthermore, it was proposed that selection against aggression may be implicated in species-specific differences in the behavior of humans and their closest extant "cousin species"—the chimpanzee and bonobo. Increased prosociality over aggression in bonobo and the rise of social tolerance in modern humans suggest that the late human evolution may have been a process of self-domestication (Brüne, 2007; Shipman, 2010; Hare et al., 2012; Hare, 2017; Wrangham, 2019).

Dog domestication began in prehistoric times, predating the development of agriculture (Morey and Jeger, 2015; Germonpré et al., 2018). There is no scientific agreement on when, where, nor how many times the dog has been domesticated but scientists agree that the dog's domestication history is complex and has been driven by a mixture of evolutionary forces (Frrantz et al., 2016; Bergström et al., 2020; Trut et al., 2021). Natural selection causes the divergence of a founder population into new populations that can exploit new and/or different environments. Once selection takes hold, the genetic differences are reinforced, as subpopulations become better adapted to specific niches. These adaptations are due to selection acting on many different traits that are important for survival and reproduction. The current data suggest that early dog domestication followed a similar scenario when predog wolves became adapted to living in proximity to humans. When humans had obtained some control over these predog populations, then artificial selection also took hold. This could act through the elimination of undesirable individuals (e.g., diseased or aggressive individuals) and/or through selection for specific behavioral, morphological, and appearance traits. The development of molecular genetic techniques further revealed the complexity of the dog's history. DNA analysis of ancient and modern dogs and wolves highlighted processes in dog domestication such as mixing dogs from different geographical regions, replacement of dog populations with other dog populations over time, and admixture of dogs with wolves (Vonholdt et al., 2010; Frrantz

et al., 2016; Bergström et al., 2020). Dog evolutionary trajectories appear to be linked to human migrations, providing a plausible explanation to a puzzle why modern dogs and ancient dogs from the same geographical regions are usually not closely related to each other (Bergström et al., 2020; Perri et al., 2021).

The intraspecific (dog−dog) and interspecific (dog−human) social behavior of the domestic dog and its progenitor species, the gray wolf, differs dramatically (Coppinger and Coppinger, 2001; Frank and Frank, 1987; Gácsi et al., 2005; Marshall-Pescini et al., 2017a; Ujfalussy et al., 2020). Although the differences in the social behavior of dogs and wolves are undeniably genetic, little is known about the evolutionary processes that led to these behavioral modifications. In the mid-20th century, the Russian geneticist, Dmitry Belyaev, argued for a primary role of selection for behavior in animal domestication. To test this hypothesis, the experimental selection of the silver fox (color morph of the red fox, *Vulpes vulpes*), a close relative of the dog, was started in 1959 (Belyaev, 1969, 1979; Trut, 1999, 2001; Trut et al., 2009). The experiment led to the development of a strain of foxes that show friendly, dog-like behavior to humans. Selecting foxes for behavior constituted a completely novel long-range approach to the genetics of behavioral modification that preceded the demonstration that dogs evolved from wolves (Vilà et al., 1997; Leonard et al., 2002; Lindblad-Toh et al., 2005). Here, we discuss these experiments after providing an overview of what is now known about the domestication and behavioral genetics of the dog.

The dog is the first domesticated species

Archeological records clearly established the dog as the first species to be domesticated but there was no consensus on the dog ancestral species until very recently. Many scientists, including Charles Darwin, proposed multispecies origin of the domestic dog (Darwin, 1868; Lorenz, 1954) and it was not until the development of DNA sequencing and genotyping methods when the gray wolf was undoubtedly established as a sole ancestor of all modern dogs (Vilà et al., 1997; Lindblad-Toh et al., 2005).

Archeological evidence for the coexistence of dogs with humans has been identified from as early as 14−17,000 years before present (ybp) in Russia, (Sablin and Khlopachev, 2002); 14,000 ybp in Germany (Nobis, 1979); and 12,000 ybp in Israel (Tchernov and Valla, 1997; Davis and Valla, 1978; Dayan, 1994). A growing number of studies identified dog remains from this time period (Pionnier-Capitan et al., 2011; Boschin et al., 2020) further supporting the hypothesis that dogs were widespread in Eurasia before the beginning of Holocene epoch (11,650 ybp). Recent studies also identified remains of dog-like canids living before the Last Glacial Maximum (26,500−19,000 ybp) in southern Siberia (Altai Mountains, Russia) dated 33,000 ybp (Ovodov et al., 2011; Druzhkova et al., 2013),

Eastern Europe (Predmosti, Czech Republic) dated 33,000 ybp (Germonpre et al., 2012, 2015), and in two sites in Western Europe: Goyet, Belguim dated 36,000 ybp (Germonpré et al., 2009) and Hohle Fels, Germany dated 40,000−35,000 ybp (Camarós et al., 2016). Although it is still debatable whether these remains belong to Pleistocene dogs and whether these domestication events were terminated by climatic changes associated with the Last Glacial Maximum, the Pleistocene remains shed light on the dog evolutionary history (Germonpré et al., 2018; Thalmann and Perri, 2018). The identification of further Pleistocene dog-like canid remains in different parts of Eurasia supports a hypothesis of multiregional dog domestication (Ovodov et al., 2011; Frrantz et al., 2016; Perri et al., 2021).

Molecular data suggest that the divergence of dogs from wolves took place at least 15,000 years ago (Savolainen et al., 2002; Skoglund et al., 2011, 2015; Freedman et al., 2014; Wang et al., 2016; Frrantz et al., 2016; Bergström et al., 2020) but several studies proposed much earlier dates (Vilà et al., 1997; Thalmann et al., 2013; Perri et al., 2021). There is still little consistency about the geographical origin of dogs, and recent DNA studies indicate that dogs originated from gray wolf populations that became extinct a long time ago (Bergström et al., 2020). Initial analyses, based on mitochondrial DNA and Y chromosome markers, suggested an East Asian origin for canine domestication (Savolainen et al., 2002; Pang et al., 2009; Brown et al., 2011; Ding et al., 2012) but later studies that included mitochondrial genomes of ancient dogs proposed a European origin (Thalmann et al., 2013). The analysis of nuclear DNA initially generated support for the Middle East origin of dog domestication (Vonholdt et al., 2010). The more recent studies that used nuclear and mitochondrial DNA markers and included both ancient and modern dogs and wolves proposed separate dog domestication events in Eastern Eurasia and Western Eurasia (Frrantz et al., 2016; Bergström et al., 2020). Studies of ancient dog remains from Americas establish the origin of New World dogs from Old World wolves (Leonard et al., 2002; Vilà et al., 1999; Vonholdt et al., 2010; Ní Leathlobhair et al., 2018) and generated additional support for the origin of dog domestication in Siberia or western Beringia during the Late Pleistocene (Ovodov et al., 2011; Perri et al., 2021). Village dogs from Africa that did not experience the population bottleneck associated with modern breed formation, and the Australian dingo and New Guinea singing dogs, were also sourced to ancient dogs domesticated in Eurasia (Boyko et al., 2009; Savolainen et al., 2004; Oskarsson et al., 2012; Ardalan et al., 2012; Larson et al., 2012).

The studies of dog ancestry also differ in their estimate of how many domestication events led to the emergence of modern dog lines (Vilà et al., 1997; Savolainen et al., 2002; Larson et al., 2012) and document admixture events between European dogs and European wolves (Vilà et al., 1997, 2003; Vilà and Wayne, 1999; Andersone et al., 2002; Vonholdt et al., 2010; Wayne and vonHoldt, 2012). Similarly, genomic traces of hybridization are

well documented in Northern American wolves with a striking example of introgression of the melanistic allele from dogs to wolves (Anderson et al., 2009; Schweizer et al., 2018). The analysis of ancient and modern dogs, however, showed that gene flow from wolves to dogs was rather limited in comparison to the gene flow dogs to wolves (Bergström et al., 2020; Gómez-Sánchez et al., 2018).

Archeological and molecular studies of dogs in the Americas provided a new window into the dog evolutionary history. The oldest confirmed dog remains dated 8−10,000 ybp were identified in Northern America, at archeological sites Koster and Stilwell II (USA, Illinois) (Morey and Wiant, 1992; Perri et al., 2019). The identification of pre-Columbian dog remains suggested the possibility of an independent dog domestication event in the Americas but this idea was later rejected by DNA analysis (Leonard et al., 2002). All pre-Columbian dogs whose remains were found outside of the Arctic Circle share a common ancestor with ancient Siberian dogs (Druzhkova et al., 2013; Ní Leathlobhair et al., 2018; Perri et al., 2021). Together, the archeological and molecular data provide convincing evidence that native American dogs arrived with human groups from Eastern Siberia through Bering Land Bridge (Beringia) that connected the two continents until ∼11,000 ybp (Olsen, 1974; Leonard et al., 2002; Witt et al., 2015; Ní Leathlobhair et al., 2018; Perri et al., 2021). The recent study of dog coprolites from archeological sites in Southwestern Illinois offered an interesting insight into the diet and parasite load of dogs and humans living in that region ∼1000 ybp (Witt et al., 2021). After Columbus came to the Americas in 1492, colonization of the new continents by European settlers had dramatic consequences for native dogs. The mitochondrial and nuclear DNA analysis showed that pre-Columbian dog lineages had vanished and were replaced by newly arrived European dogs (Castroviejo-Fisher et al., 2011; Ní Leathlobhair et al., 2018). In contrast to earlier findings (van Asch et al., 2013), recent studies found that native American dog breeds also retain very little genetic ancestry from pre-Columbian dogs and show close relatedness to modern European breeds (Parker et al., 2017; Ní Leathlobhair et al., 2018).

The genetic analysis of human and dog remains from Siberia, Beringia, and North America suggests that dogs accompanied people from Eastern Siberia to the Americas while the Bering Land Bridge still existed (Perri et al., 2021) reaffirming that dog domestication occurred at a time when modern *Homo sapiens* were still hunter-gatherers (Clutton-Brock, 1995). A long coexistence of dogs with humans is further supported by findings of genomic signatures in dogs, but not the wolf, associated with adaptation to a starch-rich diet (Axelsson et al., 2013; Arendt et al., 2016). The analysis of the amylase alpha 2B gene, an important enzyme for starch digestion, showed that selection for extra copies of this gene in the dog genome began at least 7000 ybp and was associated with the spread of prehistoric agriculture (Ollivier et al., 2016; Arendt et al., 2016). Although just how humans

and predog wolves benefitted mutually from their new-found coexistence is still an open question, several scenarios have been proposed (Lorenz, 1954; Coppinger and Coppinger, 2001; Germonpré et al., 2018).

The hypothesis of dog domestication originally proposed by Konrad Lorenz (Lorenz, 1954) and later called the Pinocchio hypothesis (Coppinger and Coppinger, 2001), suggests that sometimes, prehistoric human hunters were bringing wolf pups to their settlements. Some of these human-raised wolves were kept as adults and became founders of the domestic dog. The Pinocchio hypothesis looks attractive because it was shown that hand-raised wolves can establish strong emotional bonding with their human caregivers (Hall et al., 2015; Range and Viranyi, 2015; Lenkei et al., 2020) and keeping such animals could provide important benefits to prehistoric humans (e.g., tamed wolves could give warning signals about other predators) but this hypothesis provides an unlikely scenario of dog domestication. The Pinocchio hypothesis suggests that dog domestication began from a very small pool of gray wolves and undermines the importance of genetic selection. The dog domestication hypothesis proposed by Germonpré et al. (2018) is related to the Pinocchio hypothesis but it argues that Upper Paleolithic people were adopting wolf pups on a large scale as they valued special relationship with animals and the benefits that they provided.

The current most popular scenario of dog domestication is offered by the self-domestication hypothesis (O'Connor, 1997; Coppinger and Coppinger, 2001; Zeder, 2012). According to this hypothesis, some wolves began to explore new human-associated environments because of the attractiveness of human refuse. It is expected that only a fraction of wolves were amenable to living adjacent to humans (e.g., due to variation in stress sensitivity) and some of these wolves were likely eliminated by humans (e.g., due to increased aggressiveness). However, over generations, a population of predog wolves living comfortably in proximity to humans had appeared. Even though these wolves could be not particularly docile, they were amenable to genetic selection for human-directed social behavior that further promoted cooperation between the two species.

The key elements of the self-domestication theory are the broad genetic basis of the founding population and a gradual adaptation to living near humans. The importance of these elements was demonstrated by the Russian farm-fox experiment (Trut et al., 2009; Trut et al., 2021). Only a small fraction of wolves carried genomic variants predisposing them to develop the ability to coexist with humans; it is likely that only these genetically primed wolves could become founders of the predog population. The further adaptation of these wolves to a new human-associated niche required selection for behavior and other complex traits controlled by many genes with small effects. Genetic assimilation, increased genetic control of a trait that was originally observed in response to some environmental influence (Waddington, 1953; Crispo, 2007) could also play a role in this process. It is

unlikely that the Upper Paleolithic people purposely engaged in the effective artificial selection of the emerging predog wolf populations, therefore, it is reasonable to expect that selection for these traits was gradual and took place over a large number of generations. This genetically centered scenario of dog domestication is better supported by the self-domestication hypothesis than by the Pinocchio hypothesis.

The importance of dogs to human society is documented since prehistoric times by ancient coburial of humans with dogs (Davis and Valla, 1978) and in ancient art (Olsen, 1985; Zeuner, 1963). Wall paintings of dogs in ancient Egypt show that distinct types of dogs had already existed 2.5−4.5 000 ybp (Zeuner, 1963). The morphotypic range of such ancient dogs remains with us today among modern breeds such as mastiffs, hounds, and toy breeds, indicating that distinctly different dog types first appeared thousands of years ago (Cesarino, 1975; Harcourt, 1974; Clutton-Brock, 1981, 1995; Zedda et al., 2006; Pionnier-Capitan et al., 2011). Significantly, many of these ancient paintings and other works of art portray dogs and humans in contexts of social interaction between the two species, and evidence exists from prehistoric times for task-related interactions between humans and dogs. Dogs have been, and remain, present in almost every human culture and population as our close companions, assisting us in many tasks. These observations let to view dogs and humans as coevolved species, each influencing the development of the other (Hare et al., 2012; Shipman, 2010).

Dogs are more competent than wolves in social interaction with humans

When we talk about dogs we often describe their behavior in anthropomorphic terms. Dogs actively participate in our lives using their unique abilities to understand words from our language, the tone of our voice, and our body language (Pongrácz et al., 2001a, 2004, 2005a; Kaminski et al., 2004, 2009; Pilley and Reid, 2011; Grassmann et al., 2012). Humans and dogs have developed mutual skills for cross-species communication that led to the development of the unique social interaction between the dog and its master (Pongrácz et al., 2001a,b, 2005b; Ellingsen et al., 2010; Bray et al., 2021a; Silva et al., 2021). No other species is so routinely used as guides for blind people (Naderi et al., 2001; Serpell and Hsu, 2001) and assistants for people with other disabilities (Eddy et al., 1988; Lanea et al., 1998; Guest et al., 2006; Rintala et al., 2008).

Dogs develop an attachment to their owners after a short period of interaction, in contrast to home-raised wolves who do not show patterns of attachment to humans similar to those observed in dogs even after extensive socialization from an early age (Gácsi et al., 2001, 2004, 2005; Topál et al., 2005). Attachment is one of the basic phenomena of human social relationships (Bowlby, 1958), and one of the obvious features of the human−dog

attachment is its resemblance to the human parent−child relationship (Scott and Fuller, 1965; Topál et al., 1998, 2005; Hare and Tomasello, 2005). A recent study found that humans are particularly attracted to dog puppies that are 6−8 weeks old. This age corresponds to the weaning period when puppies' maternal support starts to decline, yet they are still too young to survive on their own. The human preference to adopt dogs at this critical point may represent an important evolutionary adaptation that facilitated dog's success as the species (Chersini et al., 2018).

The growing body of research investigating behavioral and cognitive differences between dogs and wolves stimulated a scientific debate about the origin of the dog's abilities to cooperate with humans (Hare and Tomasello, 2005; Range and Viranyi, 2015; Wynne, 2016). In the laboratory, the social skills of dogs and wolves are commonly tested using the object-choice paradigm (Soproni et al., 2001; Hare et al., 2002; Miklósi et al., 2003; Udell et al., 2008). In this test, the canid should choose between two containers using the experimenter's tap, point, or gaze cues. The correct choice is rewarded. The work of Hare et al. (2002) has shown that dogs are more skillful than hand-reared wolves at using human social cues. Dogs presented with the object-choice task showed competence in reading proximal point, proximal point and gaze, and all three cues, proximal point, gaze, and tap combined. The wolves in the same test did not follow the proximal point cue above the random chance level when presented as the only cue, but only could follow the cues when provided with combined gaze and proximal point cues together. While wolves could follow the combined cues, the number of successful trials in this test was still lower for the wolves than for the dogs (Hare et al., 2002).

More detailed, subsequent studies showed a more complex answer; wolf performance in the object-choice test is strongly influenced by the level of wolf socialization with humans and the testing environment (Miklósi et al., 2003; Virányi et al., 2008; Udell et al., 2008). Udell et al. have shown that highly socialized hand-reared wolves from a population bred in captivity can successfully use distal point cues to make a correct choice. In this particular test, the wolves tested outdoors in a familiar area outperformed dogs tested outdoors in an unfamiliar area (Udell et al., 2008). Scientists at the Eötvös Loránd University in Budapest, Hungary studied highly socialized hand-reared wolves from a different population and demonstrated that these wolves had skills to identify the correct container using tapping cues and some of these wolves were competent in reading proximal point cues (Miklósi et al., 2003; Virányi et al., 2008; Gácsi et al., 2009). Virányi et al. has found that wolves can reach a level of untrained dogs in following distal pointing gestures after several months of formal training (Virányi et al., 2008). Importantly, this study also demonstrated a variation among the hand-reared wolves in their ability to follow human cues, suggesting that there is variability in the wolves' communicative behavior, and possibly genetics,

showing the presence of the raw material in wolf populations that would be needed for selection during domestication (Virányi et al., 2008).

The demonstration that both dogs and hand-raised wolves can follow human cues in the object-choice paradigm led scientists to debate whether both dogs and wolves obtain these skills through their experience of interaction with humans or such experience is required for wolves but not for dogs. Several studies found that mother-reared dog puppies demonstrate abilities to follow human pointing cues since a very early age (6−8 weeks), and some studies indicated that these abilities do not improve with experience and age (Hare et al., 2002; Riedel et al., 2008; Gacsi et al., 2009; Bray et al., 2020, 2021b). Importantly, wolf puppies of a similar age tested in the same tasks did not show spontaneous abilities to follow human pointing cues and required extensive socialization with humans to perform above chance in the object-choice test (Salomons et al., 2021). The results of these comparative studies support the emotional reactivity hypothesis of dog domestication, postulating that dog's unique abilities to read human cues emerge spontaneously in young puppies and represent a by-product of genetic selection for tame behavior (Hare, 2017; Hare et al., 2002; Hare and Tomasello, 2005). More support for this hypothesis was also generated in the comparison of foxes selected for tameness for over 50 generations in the Russian farm-fox experiment (tame foxes) and foxes from a conventional farm-bred population that was not deliberately selected for tameness. Hare et al. (2005) found that fox kits from the tame population demonstrated abilities in reading human pointing cues similar to the abilities of dog puppies and overperformed fox kits from the conventionally bred farm foxes.

In contrast to the studies supporting the emotional reactivity hypothesis, Dorey et al. (2010) did not find evidences that dog puppies younger than 21 weeks old demonstrate abilities to follow human pointing gestures. Several studies also found that dogs' performance improves with age and experience, further indicating that socialization with humans is important for the development of skills to follow human cues for both dogs and wolves (Dorey et al., 2010; Udell et al., 2010; Jarvis and Hall, 2020). These results support the two-stage hypothesis that states that animal abilities to read human cues depend on (1) the animal's acceptance of a human as a social partner and (2) the animal's level of conditioning to follow human limbs (Udell et al., 2010; Wynne, 2016; Jarvis and Hall, 2020). According to the two-stage hypothesis, one can argue that dog puppies and tame fox kits are genetically predisposed to establish social interaction with humans, and due to this prohuman sociality, they have better abilities to learn human behavior during a short period of time. A recent study by Lazzaroni et al. (2020) demonstrated that differences between adult dogs and wolves in wiliness to interact with humans can impact the results of their performance in human-cooperation tasks further demonstrating the importance of human acceptance as a social partner for canid abilities to interact with humans.

The number of studies investigating different aspects of the cognitive abilities of dogs and wolves has been growing dramatically since the early 2000s. Several studies have shown that dogs are competent in the recognition of human attention status (Call et al., 2003; Gácsi et al., 2004; Bräuer et al., 2004). Dogs use the orientation of the body, the orientation of the head, and the visibility of the eyes to distinguish between "attentive" and "inattentive" humans (Gácsi et al., 2004; Virányi et al., 2004). Dogs are more likely to beg for food from a person who can see them than from a person whose vision is obscured by a blindfold or a book (Cooper et al., 2003; Gácsi et al., 2004; Udell et al., 2011). Comparison of dog behavior across several tests led to the conclusion that dogs can rely on the same set of human facial cues that are used by humans to understand the attention status of other humans (Gácsi et al., 2004).

Dogs also look to humans for assistance and direction more than wolves. When human-reared dogs and wolves trained to solve a simple manipulative task were presented with an impossible version of the same test (e.g., opening a locked box with food inside), dogs looked at the human almost immediately and then directed their gaze between the human and the box, while wolves did not look to the human or did it for a much shorter time period while continuing to attempt to solve the problem on their own (Brubaker et al., 2017; Marshall-Pescini et al., 2017b; Miklósi et al., 2003; Salomons et al., 2021; Udell, 2015). These findings support the idea that a dog's inclination to look at a human's face is one of the key differences between dogs and wolves in initializing and maintaining communicative interaction with humans (Miklósi et al., 2003) but these results can also have a different explanation (Marshall-Pescini et al., 2017b; Udell, 2015). Udell (2015) found that wolves demonstrate higher persistence in an unsolvable task and spend more time investigating a puzzle box in both human-present and alone conditions than dogs do. Marshall-Pescini et al. (2017b) showed that accounting for the time that wolves and dogs spend in physical contact with an object can explain differences in the amount of gazing time during the test. These findings indicate that an unsolvable task test demonstrates not only differences in human-oriented social responses of dogs and wolves but also differences in problem-solving behavior of the two species.

Differences in the behavior of dogs and wolves in social interaction with humans are recognized early in the postnatal period. The amount of interaction required for the socialization of a wolf is dramatically different from and greater than the amount of interaction required for the socialization of a dog (Coppinger and Coppinger, 2001). Successful socialization of wolf puppies involves the puppy's separation from the family before the eyes open and intense socialization with a human caregiver. The competence of dog and hand-reared wolf puppies in their interaction with humans was tested in multiple tests starting at 3 weeks old (Gácsi et al., 2005; Ujfalussy et al., 2020). Significant differences in their behavior have been recorded at each

time point. Dog puppies showed more communicative behavior with humans. Wolf puppies were more often aggressive to humans and more often demonstrated avoidance behavior (Gácsi et al., 2005; Ujfalussy et al., 2020). The differences in behavior of hand-raised wolves and domestic dogs persist into adulthood, clearly demonstrating that thousands of years of coexistence with humans led to the appearance of novel behavioral skills in dogs.

Analysis of temperament traits in dogs

Scientists often refer to dogs when they discuss the remarkable variation in size that can be observed within a single mammalian species. However, the spectrum of behaviors demonstrated by dog breeds is not less amazing. Although reliable historical records on breed origins barely exist for most breeds, there is substantial evidence that breed formation was associated with cultural and economic developments in human societies (Clutton-Brock, 1995; Willis, 1995). Modern breeds were officially registered relatively recently, less than 200 years ago, but a gamut of breeds recognized for their unique behavioral skills had existed for a much longer period of time (Leighton, 1910). Dogs with similar working skills but differences in terms of the specific functions they served, as well as differences in appearance, were recognized as independent breeds and received official breed standards when registries were created by dog clubs (Dalziel, 1897; American Kennel Club, 2006). The migration of Europeans and their dogs to the New World promoted the formation of new working breeds descended from the old breeds or their crosses, incorporating new combinations of desirable skills that were better adapted to new needs and places (Clark, 2003; Cummins, 2002; Huson et al., 2012). Currently, there are over 350 breeds recognized worldwide and an application of genomic tools has started to reveal the history of breed formation (Vonholdt et al., 2010; Parker et al., 2017).

A recent study analyzed the genetic relationship among 161 dog breeds from different parts of the world and identified 23 breed groups (genetic clades) (Parker et al., 2017). The breeds within identified clades were found to share common behaviors, physical appearance, and/or geographic origin. In general, the analysis of genetic relatedness identified hybridization among breeds within clades but little admixture between breeds from different clades, suggesting that identified clades represent dog breed types that existed before the creation and registration of modern breeds. Dog breed type formation was likely linked to the development of new human activities and migrations. Several breed clades were found to have similar behavioral functions suggesting that selection for similar behaviors could take place in different geographical regions independently (Parker et al., 2017).

Selection for specific behaviors has clearly played an important role in the course of the formation of the working breeds of dogs. The development of herding breeds is inevitably linked to the growth of livestock farming.

Herding breeds differ among geographical regions, as well as the species they herd and the types of tasks they perform (Combe, 1987). Genetically, working sheepdog breeds that originated in Great Britain are more related to each other than to any other breed category selected for herding or different working tasks in Great Britain or other countries (Parker et al., 2007; Vonholdt et al., 2010). Similarly, small Hungarian sheepdogs form a genetic clade that is distinct from other breed clades (Parker et al., 2017).

The use of dogs for hunting is reflected in paintings and historical records from ancient times (Columella, 1954; Clutton-Brock, 1995). Although we do not have evidence that modern hunting breeds are descendants of these ancient dogs, it is instructive to note that the sighthound breeds, perhaps the oldest type of hunting dogs, are genetically closely related to each other (Vonholdt et al., 2010; Parker et al., 2017), even though from different geographical locations. Likewise, hunting breeds sharing highly specialized behavioral repertoires, such as retrieving breeds, are more closely related genetically to each other than to breeds, such as scent hounds, selected for different hunting skills (Parker et al., 2017).

One of the most comprehensive studies of dog behavior was conducted at Jackson Laboratory in Bar Harbor in the mid-20th century (Scott and Fuller, 1965). The experiment was designed to gain insight into the genetics of social behavior; the dog was selected as a model because dog social behavior was regarded as resembling human behavior, and there was a large amount of variation in behavior among the dog (Scott and Fuller, 1965; Hahn and Wright, 1998). The five dog breeds studied in Bar Harbor (Basenji, Beagle, Cocker Spaniel, Shetland Sheepdog, and Wire Haired Fox Terrier) belong to different breed clades and historically were selected for different working skills (Parker et al., 2017). The different aspects of the behavior of these breeds and their crosses were studied in great detail from birth to approximately 1 year old. The study in Bar Harbor lasted for 13 years and has led to many insights, not only on our understanding of dog behavior, but on other disciplines including human psychology, genetics, and evolutionary biology. Here, we describe a few experiments conducted in Bar Harbor, which demonstrate the remarkable variation in behavior among dog breeds, the complex genetic inheritance of these behavioral differences, and the importance of both genetic and environmental factors for behavior development.

A detailed analysis of canine behavioral development from the early postnatal period identified a critical period in dog socialization (Freedman et al., 1961). The opening of the socialization period takes place when sensory and motor abilities are sufficiently mature that puppies can actively interact with each other and their surroundings (Scott, 1962). This period's gradual closure begins near weaning, when puppies are 7–10 weeks old (Scott, 1974; Morrow et al., 2015). The socialization period is called a critical period because experiences obtained during this developmental stage are important for the formation of foundational assumptions about the environment,

gaining experiences, and shaping expectations in social interactions (Scott and Fuller, 1965; Scott et al., 1962, 1974). For example, puppies raised in an enclosure with their mothers but without human exposure during the socialization period will avoid human handlers later in life (Freedman et al., 1961). The role of the environment during this period was also shown to be important for the development of dog working behaviors, for example, herding behavior (Coppinger and Coppinger, 2001).

Analysis of social relationships among littermates revealed different behavioral schema of aggression. By 11 weeks old, 95% of litters developed a food-related dominance hierarchy. Fox terriers formed more dominant relationships than cocker-spaniels or beagles. Significantly more male fox terriers and basenjis formed dominance relationships than did females, whereas beagles and cocker-spaniels did not show gender-specific differences in dominance relationships. Shelties (Shetland sheepdogs) developed strong territorial rather than food-related dominance relationships, whereas basenjis showed the opposite pattern: absence of territorial aggression but escalated aggression over food (Scott and Fuller, 1965; Hahn and Wright, 1998).

Measuring emotional reactivity showed significant differences among the breeds: terriers, beagles, and basenjis were consistently more emotional than shelties and cockers. Although significant differences in breed reactivity were observed, a variation in reactivity was also very apparent among individuals from the same breed. The reactivity showed very little change with age and no differences between males and females.

Problem-solving behavior was evaluated in different tests including a maze test. Fox terriers and shelties made more errors and required a longer time in moving through the maze. Basenjis had the best time on the first day of learning but beagles appeared superior on overall scores through multiple trials. The authors suggested that the beagle's success was attributable to their avoidance of stereotyped habits and random exploration of their surroundings.

Differences in trainability were observed among breeds and individuals from an early age. Some individuals showed response to training at 5 weeks old (forced training to be quiet on the weight platform); by the age of 16 weeks, nearly 70% of the cocker spaniels were remaining quiet in the test while only 10% of the fox terriers were. All dogs learned to walk appropriately on a leash within a 10 days training period but different breeds differed in the course of their learning: basenjis were outstanding in their vigorous resistance to restraint, shelties interfered excessively with the handler by leaping on him and winding between his legs; and beagles demonstrated excessive vocalization during the initial stages of training. These experiments demonstrated that the basic trainability characteristics of the different breeds tend to be specific to particular test situations and that they are based on a large variety of capabilities. The authors concluded "*In short, the effect of heredity upon trainability is highly complex, both because of the number of specific basic abilities involved and because of the complicated interaction between them made possible by behavioral adaptation*" (Scott and Fuller, 1965).

Genetic inheritance of several behavioral characteristics was demonstrated by experimental cross-breeding among breeds with distinctive behavioral repertoires. Fearful behavior was analyzed in crosses between basenjis (all tested individuals exhibited some fearful behavior) and cocker-spaniels (62% of tested individuals exhibited fearful behavior). F1 dogs exhibited behavior broadly similar to that of the basenji parents, although the inheritance of different specific behavioral characteristics in this cross varied significantly. In several training and spatial-orientation tests, the F1 offspring were superior relative to both parents suggesting a complex interplay in the inheritance of different behavioral characteristics.

Furthermore, this study clearly demonstrated the influence of genotype-by-environment interaction on dog behavior. As summarized in Houpt, 2007: "*The most intriguing result was the interaction of genotype and experience* (Freedman, 1958). *Puppies were raised in isolation and exposed to either tolerant humans or disciplinary humans who made the puppies sit and wouldn't play with them. Later the puppies were presented one by one with a bowl of food. If they approached, the handler would clap his hands to frighten them away. The handler then left the room so the puppies were free to eat the food. The basenjis did, no matter how they had been handled. The Shetland sheepdog never did, no matter how they had been handled. The disciplined beagles ate the food, but not the tolerantly treated ones. The conclusion is that identical handling will have different effects on different genotypes of dogs*."

The Bar Harbor experiment clearly shows the complexity of dog social behavior in terms of its development and inheritance. A strong maternal effect was shown for several behavioral characteristics. In particular, the experiment demonstrated how easily behavior can be misjudged in terms of its genetic origin or gene-environment interaction nature (Scott and Fuller, 1965). Underestimation of critical parameters underlying the formation of dog behavioral traits can lead to serious pitfalls in an attempt to identify genetic regulation of these behaviors.

These days, the majority of dogs are acquired as family pets. Each dog breed has a strictly defined standard and breeding is often much more focused on selection for morphologic and appearance traits than on behavior. In fact, many behaviors for which working breeds were originally selected became inconvenient in modern life and are not supported by current breeding practices. Behavioral characteristics for which the breeds were known historically thus often do not correspond to the behavioral characteristics of these breeds today. For example, Kubinyi et al. analyzed temperament traits in different breeds in Europe and found significant differences for some breeds between behavioral characteristics provided by the breed standard and behavioral profiles of the same breeds based on the owners' reports (Kubinyi et al., 2009).

In the Kubinyi et al. (2009) study, 93.3% of the respondents representing 14,004 dog owners indicated the "family member" category as the function

of their dogs. These results are consistent with other studies in Europe, Australia, and the United States indicating that the majority of dog owners acquire dogs not for specific working tasks but for companionship (Bennett and Rohlf, 2007; Jagoe and Serpell, 1996; Serpell, 2004; Ellingsen et al., 2010). Although the strict demand for working skills is reduced, some breed clubs are making an effort to preserve these skills through behavioral tests and incorporating the results of these tests into breeding programs. This sometimes leads to the development of two independent lines of breeding, one for show and pet dogs, but another one for working dogs, as in, for example, the Border Collie and Labrador Retriever breeds (Fadel et al., 2016; Ilska et al., 2017).

Programs implemented by organizations to evaluate specific aspects of dog behavior (e.g., herding, protection, detection of drugs or explosives) or for general behavioral assessment (e.g., to screen potential breeding populations for dogs with undesirable behavioral characteristics), have yielded valuable data for the scientific evaluation of dog temperament. Svartberg and Forkman (2002) identified five factors (dimensions) underlying canine temperamental traits using data from 1175 dogs of 47 different breeds tested in the dog mentality assessment (DMA) test designed by the Swedish Working Dog Association (Svartberg and Forkman, 2002). The factors were labeled as "Playfulness," "Curiosity/Fearlessness," "Chase-proneness," "Sociability," and "Aggressiveness" and accounted for 37% of common variation in the test. The first four factors were related to one other higher-order factor, which was interpreted as shyness−boldness. The same broad factors with slight variations were identified in an analysis of 15,329 dogs from 164 breeds performed for each of eight Fédération Cynologique Internationale (FCI) breed groups (Svartberg and Forkman, 2002). These data argue that personality factors underlying dog behavior in the DMA test are universal among breeds and not related to specific characteristics of FCI breed groups. Consistency of these behaviors over repeated tests for individual dogs was observed (Svartberg et al., 2005). A heritability of 0.25 for the shyness-boldness dimension was reported (Saetre et al., 2006).

Another common approach for assessment of dog behavior is the collection of information from dog owners by questionnaires (Serpell and Hsu, 2001; Hsu and Serpell, 2003; Svartberg, 2005; Bennett and Rohlf, 2007; Vas et al., 2007; Kubinyi et al., 2009; Ellingsen et al., 2010; Arhanta et al., 2010; Salonen et al., 2021). This approach assumes that the dog owner knows its dog's typical behavior and that by asking appropriate questions this information can be extracted in a reasonably accurate, quantitative, and reliable manner (Serpell and Hsu, 2001; Hsu and Serpell, 2003). The study by Kubinyi et al. (2009) cited above used a 48-item questionnaire adapted for the dog from a questionnaire for human personality assessment. Factor analysis on the dataset including 14,004 dogs identified four dimensions of dog personality: calmness, trainability, sociability, and boldness. Each dimension was

shown to be influenced by other variables: calmness was influenced by age, neutered status, and the number of professional training courses a dog experienced; trainability was influenced by training experience, age, and the reason for keeping the dog; sociability to other dogs was mainly determined by age, sex, training experience, and time spend together; boldness was affected by the sex, age of the dog, and the age of the acquisition (Kubinyi et al., 2009). Differences in trainability and boldness among the dog groups were identified: herding dogs were more trainable than hounds, working dogs, toy dogs, and nonsporting dogs, while terriers were bolder than hounds and herding dogs (Turcsán et al., 2011).

Currently, one of the most commonly used questionnaires in dog research is C-BARQ, which was originally designed and validated for evaluating the behavior and temperament of guide dogs (Serpell and Hsu, 2001). Using 132 items from a questionnaire filled by owners of 2054 dogs, Hsu and Serpell identified 11 factors that accounted for 57% of common variance in the scored items (Hsu and Serpell, 2003). The factors have been labeled based on the most significant traits contributing to each factor. The factors identified by Hsu and Serpell (2003) arguably describe narrower behavioral categories than the factors identified in the study by Svartberg and Forkman (2002). For example, the study by Hsu and Serpell identified three factors related to aggression: stranger-directed aggression, owner-directed aggression, and dog-directed fear or aggression.

The C-BARQ data collected for thousands of dogs of different breeds have been recently used for genetic studies of dog behavior (Zapata et al., 2016; MacLean et al., 2019). MacLean et al. (2019) estimated among-breed heritability for 14 C-BARQ factors in a dataset including 101 breeds. The stranger-directed aggression and trainability were found to have the highest heritability (variance of these traits attributable to the genetic similarity among breeds) while fear behavior had the lowest heritability. The genome-wide association study identified 131 genetic markers significantly associated with behavioral differences among breeds that were largely located in genes known to be expressed in the brain (MacLean et al., 2019). Zapata et al. (2016) used a similar approach and found that some genomic regions implicated in morphological differences among dog breeds are associated with aggression and fear-related traits.

Several recent studies focused attention on variation in behavior within breeds (Persson et al., 2015; Ilska et al., 2017; Friedrich et al., 2019; Sarviaho et al., 2019). Ilska et al. (2017) collected C-BARQ data for 1975 pedigreed Labrador Retrievers from the United Kingdom (UK) while Friedrich et al. (2019) collected C-BARQ data for 426 pedigreed UK German Shepherds (GSD) that were privately owned in the UK and 615 pedigreed GSD from the Swedish Armed Forces. A comparison of the two studies shows differences in heritability of the C-BARQ factors between the two breeds. For Labrador Retrievers, the factors with the highest heritability were chasing,

stranger-directed aggression, and nonsocial fear. Authors also used a modified analysis of C-BARQ data and found heritability >0.2 for such traits as fetching, noise fear, nonowner-directed aggression, trainability, unusual behavior, and agitated behavior (Ilska et al., 2017). In contrast, the GSD study found heritability >0.2 only for human-directed playfulness and low heritability for stranger-directed interest and nonsocial fear. The rest of the factors in GSD showed heritability close to zero (Friedrich et al., 2019). The results of these studies indicate that the genetic composition of dog breeds is related to the heritability of behavioral traits and support results of previous studies that demonstrated complex genetic inheritance of dog behavior (Scott and Fuller, 1965; Krushinskii, 1960). However, it is also important to note that the expression of behavioral traits can be influenced by environmental factors (e.g., Labrador Retrievers and GSD can be handled differently by their owners), and these factors can affect heritability estimation.

A group at Linköping University, Sweden, analyzed the behavior of 498 research beagles in an unsolvable problem task (Persson et al., 2015). They observed moderate heritability of two traits: test interactions (interactions with the test device) and social interactions (interactions with an experimenter) and almost zero heritability for eye contact and physical contact. Females had significantly higher scores for social interactions and physical contact with an experimenter. A genome-wide association study (GWAS) of tested dogs identified a region on chromosome 26 that was significantly associated with physical contact (Persson et al., 2016). In the follow-up study, Persson et al. (2018) used a different cohort of dogs of different breeds (Golden Retrievers and Labrador Retrievers) tested in unsolvable problem tasks and these findings. These results provide a strong indication that a region on dog chromosome 26 that contains genes associated with autism and other human behavioral conditions is implicated in human-directed social behavior in dogs (Persson et al., 2016, 2018).

Sarviaho et al. (2019) studied 330 Finish GSD whose behaviors related to fear of loud noises and fear of strangers or novel situations were measured using an owner-completed behavioral questionnaire. Previous studies reported moderate heritability of these traits in GSD (van der Waaij et al., 2008; Friedrich et al., 2019). The GWAS found a significant association between the fear of loud noises and a region on chromosome 20 and a significant association between the fear of strangers and novel situations on chromosome 7. The loci identified by Sarviaho et al. (2019) did not overlap with the results of GWAS conducted by Friedrich et al. (2019) in the cohort of the UK and Swedish GSD. These differences could be due to many reasons but further validation of identified loci is needed.

Assessment of dog behavioral phenotypes using data from tests conducted either by dog organizations or by owner-filled questionnaires has some pitfalls (for review see Jones and Gosling, 2005; Hall and Wynne, 2012). The former, biased toward dog populations whose owners are

interested in dog training, is often focused on specific behavioral characteristics and targets a limited set of breeds. The latter do not provide results of direct observation of dog behavior in a defined period of time and are sensitive to both owner experience in judging dog behavior and the validity of the questionnaire. There are limitations in this observational research, from the logistical difficulties in observing dogs in their owners' homes to the differential effects that testing dogs in research labs can have on different dogs, depending on how they react to the novel environment. The rapid development of video and internet tools offers a new opportunity for collecting behavioral data (Stewart et al., 2015). Gnanadesikan et al. (2020) collected data for 1508 dogs representing 36 breeds through a citizen science website Dognition, which asks owners to test their dogs in a series of behavioral and cognitive tests. Estimation of among-breed heritability of behaviors scores obtained in these tests was performed following MacLean et al. (2019) and identified moderate-to-high heritability for two factors: inhibitory control and communication (Gnanadesikan et al., 2020). The citizen science approach allows testing of dog behavior in a familiar environment by a familiar person and therefore it eliminates some variables that can significantly affect dog performance. It can be predicted that the growth and availability of digital technologies will advance the collection of dog behavioral data around the globe.

Multiple efforts have adopted a candidate gene approach to identify genes associated with behavioral traits in dogs (reviewed in Hall and Wynne, 2012). Positive associations were identified for many behavioral traits and candidate genes including activity-impulsivity with *DRD4* and *TH* in German Shepherds and Siberian Husky and with *DAT*, *DBH*, *DRD4* in Belgian Tervuren (Hejjas et al., 2007, 2009; Kubinyi et al., 2012; Wan et al., 2013); activity level with *SLC1A2* and *COMT* in Labrador Retrievers (Takeuchi et al., 2009a); circling behavior in Belgian Malinois with *CDH2* (Cao et al., 2014); human-directed aggression with *DRD1*, *HTR1D*, *HTR2C*, and *SLC1A1* in English Cocker Spaniels (Våge et al., 2010); aggression with *SLC1A2* in Shiba Inu (Takeuchi et al., 2009b), but with *AR* in Akita Inu (Konno et al., 2011); human-directed social behavior with *OXTR* and *OPRM1* genes in several breeds (Kis et al., 2014; Kubinyi et al., 2017; Persson et al., 2017). Most of the candidate gene studies were performed using a relatively small number of dogs and only a few were validated in independent data sets (Kubinyi et al., 2012; Wan et al., 2013). In contrast, the oxytocin receptor (*OXTR*) gene was studied by several research groups using different dog populations (Kubinyi et al., 2017; Persson et al., 2017; Konno et al., 2018). The validity of a single gene approach may be questionable due to the complex genetic architecture of dog behavior, but results obtained for the *OXTR* gene suggest a potential role of oxytocin system in dog−human interaction (Kikusui et al., 2019; Marshall-Pescini et al., 2019; Trut et al., 2021).

The oxytocin system is involved in different aspects of social behavior in mammals including mother–offspring attachment, pair bonding, and social recognition (Bosch and Young, 2018). Under the assumption that domestication modified two behavioral dimensions: fear response and human-directed social behavior (Trut et al., 2009, 2021; Wheat et al., 2019) the former was considered to be linked to changes in the hypothalamic–pituitary–adrenal axis and the latter to changes in the oxytocin system (Buttner, 2016; Kikusui et al., 2019). Romero et al. (2014) showed that nasally administered oxytocin increased dog affiliative behavior toward their owners and dog partners. Furthermore, it was shown that interspecies contacts increased urinary oxytocin levels in dogs, and gazing behavior from dogs increased urinary oxytocin concentrations in humans (Nagasawa et al., 2015). The same effects were not observed for hand-raised wolves. The follow-up studies showed different effects of oxytocin treatment on dog behavior, and some were inconsistent with earlier findings (Kovács et al., 2016; Thielke et al., 2017; Barrera et al., 2018; Wirobski et al., 2021). Variations in methodologies, breed and gender differences, and different *OXTR* genotypes of tested animals were suggested among factors that could lead to inconsistency among these studies (Marshall-Pescini et al., 2019; Persson et al., 2017; Kis et al., 2014). Persson et al. (2017) found that nasally administrated oxytocin had significant effects on contact-seeking behavior only in dogs with particular *OXTR* genotypes indicating that these genotypes could be under selection in some dog populations but not in others.

Several genetic studies identified genomic regions that differentiate dogs from wolves and show signals of selection in dog genomes. The analysis of these regions identified genes implicated in morphological, appearance, and metabolic traits in dogs and candidate genes for behavioral differences between the two species (Vonholdt et al., 2010; Axelsson et al., 2013; Wang et al., 2013; Freedman et al., 2016; Cagan and Blass, 2016; Plassais et al., 2019). One genomic region that differentiates dogs from wolves is homologous to the region on human chromosome 7 (Vonholdt et al., 2010), where a deletion causes Williams–Beuren syndrome in humans, a complex disorder associated with exceptional gregariousness (Doyle et al., 2004; Jarvinen-Pasley et al., 2008). These findings suggested that this region may harbor genes influencing social behavior in both humans and dogs. The follow-up genetic analysis of dogs and wolves with known behavioral phenotypes has found that structural variants in *GTF2I* and *GTF2IRD1* genes located in the human Williams–Beuren syndrome region were associated with extreme sociability in dogs (vonHoldt et al., 2017). Establishing direct connections between identified genomic regions and behavioral traits in dogs is a difficult task. As dogs diverged from wolves so long ago and have been selected for so many different traits, one would certainly expect their genomes to harbor multiple species-specific selection signals not necessarily associated with behavioral differences. Similar to the Williams–Beuren syndrome region

finding, comparative studies with other species can help to highlight genomic regions and gene pathways implicated in selection for behavior. The synergistic opportunities for genetic analysis of behavior are provided by the Russian farm-fox experiment that recapitulated canine domestication in the silver fox.

The farm-fox experiment

The silver fox is a coat color morph of the red fox (*Vulpes vulpes*). In contrast to the dog that was domesticated prehistorically, the fox was domesticated in controlled farm conditions. Experimental domestication of farm-bred foxes was started by Dmitry Belyaev and Lyudmila Trut at the Institute of Cytology and Genetics (ICG) of the Russian Academy of Sciences, in Novosibirsk, Russia, in the 1950s and is still ongoing, as has been reviewed in several publications (Trut, 1999, 2001; Trut et al., 2004, 2009) and in the book "How to tame a fox" by Lee Dugatkin and Lyudmila Trut. Belyaev proposed that selection for behavior was the primary force in the course of animal domestication. He further hypothesized that selection for unusual behavior in wild species, that is, a tame response to humans, may affect many physiological processes in animals under selection (i.e., stress-response and seasonality of reproduction) and lead to the emergence of variation in morphology and appearance, common features of domesticated species (Belyaev, 1979; Trut, 1988; Belyaev and Trut, 1989). The fox was selected as a model to test this hypothesis.

When the fox domestication experiment began, foxes had been bred in captivity for over 50 years. Fox fur farming was pioneered on Prince Edward Island (PEI) in Southeastern Canada, beginning in the 1890s (Westwood, 1989). The success of the fox industry's pioneers on PEI promoted the development of fox ranches in other Canadian provinces and Alaska in the early 1900s (Forester and Forester, 1973). These farms used wild-caught foxes as breeding stock, some of which were later mixed with the PEI stock (Hodgson, 1947). Farmers primarily raised the silver-black coat color variant of red foxes, which had the greatest economic value and were subsequently used to stock fur farms in many areas of North America and Eurasia (Petersen, 1914; Westwood, 1989; Nes et al., 1988; Bespyatih, 2009). The analysis of mitochondrial DNA and Y-chromosome markers identified Eastern Canada as the primary source of ancestry for farm-bred fox populations maintained at the ICG in Novosibirsk (Statham et al., 2011; Rando et al., 2017).

Selection of foxes for tame behavior

In the beginning of the experiment, the behavior of several thousand foxes from several farms across the former USSR was screened. Most conventional

foxes showed aggressive and fear-aggressive behavior to humans but ~10% were calmer, although avoided an interaction (Belyaev, 1979; Trut et al., 2020). Belyaev and Trut selected 130 such foxes as founders for the experimental population (Trut, 1999). The response to human presence was the sole selection criteria during the further selection of this population for tame behavior. At first, the selection was focused against aggressive responses to humans, as summarized in Trut et al. (2004): "*The main task at this stage of selection was eliminating defensive reactions to humans. In order to reveal variability in the expression of these reactions more completely, the animals in the selected population were subjected to more intensive contacts with humans than in usual practice. During these contacts, the pups were subjected to a number of tests: the experimenter attempted to hand feed, stroke or handle them. This type of human–animal communication continued for the first three to four months of life of the animals. As a result, the emotionally negative defensive reactions to humans in these foxes weakened, disappeared or, in some of the animals, emotionally positive reactions were formed. The foxes that retained aggressive–fearful reactions to humans in spite of the 3-month period of human contacts with them, were eliminated by selection from the population as soon as in 2 to 3 generations* (Trut, 1980a,b, 1999). *In generation 4 of selection, the first pups appeared that did not form aggressive–fearful reactions to humans as a result of positive contacts with them. On the contrary, these pups demonstrated emotionally positive response to humans: when the experimenter approached them, they whined and wagged their tails anticipating a positive contact*." Results of the selection against defensive aggression became apparent after a few generations of selective breeding. Subsequently, selection for an emotionally positive ("friendly") response to humans was applied. The behavior of all foxes was evaluated in a test with five consecutive stages: (1) observer approaches the cage; (2) observer stands near the closed cage; (3) observer opens the cage door and stands nearby; (4) observer attempts to touch the fox; (5) observer closes the cage door, then stays near the closed cage (Trut, 1980a; Trut et al., 2004). Fox behavior in this test was scored based on their response to humans using a categorical system (Table 8.1). The behavior of young animals was tested several times during development, and the final test was given to all pups at 7–8 months old. Only the tamest individuals in each generation, less than 10%, were bred. At the same time, a deliberate effort was made to avoid inbreeding (Trut, 1999, 2001; Trut et al., 2004; Kukekova et al., 2004). The response to selection was extremely rapid (Table 8.2). Improvement of behavioral scores recorded in fox pedigrees clearly showed genetic inheritance of tame behavior. It is important to note that except for the first few generations of selection, foxes from the population selected for tame behavior did not receive special handling. All human

TABLE 8.1 System for scoring behavior in tame fox population and selecting the most tame foxes for the breeding program.

Animal reaction	Scores
Passive-protection response; fox avoids experimenter or bites if stroked or handled, come if offered food.	0.5–1.0
Foxes let themselves be petted and handled, but show no emotionally friendly response to the experimenter.	1.5–2.0
Foxes show emotionally-positive, friendly, response to the experimenter, wagging tails, and whining.	2.5–3.0
Foxes are eager to establish human contact, whimpering to attract attention and sniffing and licking experimenters like dogs. They start displaying this kind of behavior before 1 month postnatal age.	3.5–4.0

Tame behavior is scored from zero (representing "neutral" behavior; an absence of both active aggressive and tame responses directed toward the observer) to 4 (representing the most tame behavior).
Source: From Trut, L.N., 1980a. The genetics and phenogenetics of domestic behaviour. In: Problems in General Genetics (Proceeding of the XIV International Congress of Genetics), vol. 2, book 2, pp. 123–136.

TABLE 8.2 The number and proportion of elite-behavior progeny at different stages of selection.

Year of study (generation of selection)	Number of progeny scored	Out of them, elite animals	
		Number	Proportion %
1965 (F_6)	213	4	1.8
1970 (F_{10})	370	66	17.8
1980 (F_{20})	1438	503	35.0
1990 (F_{30})	1641	804	49.0
2002 (F_{42})	902	642	71.2

Source: From Trut, L.N., Pliusnina, I.Z., Oskina, I.N., 2004. An experiment on fox domestication and debatable issues of evolution of the dog. Genetika (Russ.) 40, 794–807.

contacts were, and continue to be, limited to maintenance and testing procedures. Foxes from the tame population show a friendly response to humans as early as 1-month postanatal and remain friendly throughout their entire lives. Tame foxes are eager to establish human contact, whimper to attract attention, and sniff and lick similar to dogs (Fig. 8.1A).



(A) (B)

FIGURE 8.1 (A) Behavior of foxes from the tame population. (B) Behavior of a fox from the aggressive population.

Selection of foxes for aggressive behavior

In parallel with selection for tameness, selective breeding of farm foxes for aggressive behavior began in the 1970s. Because there is deliberate selection on commercial farms against animals that show excessively aggressive responses to humans, selection of the aggressive strain at ICG was undertaken to preserve this behavior for research. Fifty farm-bred silver foxes with the most aggressive responses to humans were selected from several fox farms and used as founders of the aggressive population. The critical distance between experimenter and caged animal and intensity of fox aggressive response was the major criterion for measuring aggression in the fox population and selecting animals for producing the next generation (Table 8.3). Progress in selection for aggressive behavior did not follow the same pattern as selection for tame behavior but was slower (Trut, 1980a,b). Foxes from the aggressive population are, however, consistently and distinctly aggressive toward humans and difficult to handle (Fig. 8.1B).

Behavioral and physiological changes associated with selection for tame behavior

The genetic basis of tame and aggressive fox phenotypes has been clearly demonstrated in multiple experiments that included experimental cross-breeding of tame and aggressive animals, cross-fostering of newborn pups, and embryo transplantation (Kukekova et al., 2008, 2011; Trut, 2001; Trut, 1980a; Trut, 1980b). Although selection of foxes for tame behavior was strictly limited to defined behavioral criteria, further developmental and behavioral differences emerged in tame foxes. Opening of the eyelids and the external auditory canal was accelerated; the sensitive period for socialization persisted past 60 days old (compared to less than 45 days in unselected foxes); and play activity (normally only seen in infantile wild type foxes) extended into adulthood (Belyaev et al., 1985; Plyusnina et al., 1991; Trut, 2001). Tame foxes also developed a

TABLE 8.3 System for scoring behavior in aggressive fox population and selecting the most aggressive foxes for the breeding program.

Animal reaction:	Scores:
Fox shows teeth, snarls, growls *at first sight of a human*. When the experimenter is near a *closed cage* fox attacks the experimenter and other objects in the field of view. Bared teeth and fixed dilated pupils.	−4.0
When the experimenter is near a *closed cage*, the fox shows teeth, snarls, growls, tries to attack both the experimenter and other objects in the field of view. Bared teeth and fixed dilated pupils.	−3.5
When the experimenter is near an *open cage*, the fox shows teeth, snarls growls, and attacks the experimenter and other objects in the field of view. Bared teeth and fixed dilated pupils.	−3.0
When the experimenter is near the *open cage*, the fox growls but does not attack.	−2.5
When the experimenter, near the *open cage*, moves the protected arm toward the fox, it growls and tries to bite.	−2.0
As the experimenter opens the cage fox is calm, but attempts to touch the fox provoke it to show teeth and snarl.	−1.5

The score is based on the critical distance between the experimenter and the caged fox when the animal first demonstrates an aggressive reaction to the experimenter's presence. Originally the scoring system was based on a range from 0 (least aggressive) to −4 (most aggressive). However, after multiple generations of selection, no current individuals exhibit behavior scoring between −1.5 and 0.
Source: From Trut, L.N., 1980a. The genetics and phenogenetics of domestic behaviour. In: Problems in General Genetics (Proceeding of the XIV International Congress of Genetics), vol. 2, book 2, pp. 123–136.

novel repertoire of vocalizations toward humans (Gogoleva et al., 2009, 2011). Significant differences in corticosteroid levels and significant differences in hypothalamic–pituitary–adrenocortical system reactivity in ontogenesis were observed between tame and conventional foxes (Oskina and Tinnikov, 1992; Trut and Oskina, 1985; Oskina, 1996). Significantly lower density of serotonin 5-HT_{1A} receptors was observed in the hypothalamus, and significantly higher levels of serotonin and tryptophan hydroxylase were detected in the midbrain and hypothalamus of tame foxes (Popova et al., 1997; Popova, 2006; Trut, 2001). A transformation of the reproductive seasonality also was observed: some foxes from the tame population showed sexual activity outside of the regular breeding season, and a few females have been mated twice in a year (Trut, 1980b; Belyaev and Trut, 1983).

Several de novo traits were detected in the fox population selected for tameness. In particular, coat color changes such as the appearance of a white spot on the head (Star phenotype) and loss of pigment in other areas began to appear in the eighth selected generation (Belyaev et al., 1981). It is

intriguing that white spotting, which appeared without direct selection or inbreeding in the tame fox population, is frequently observed as a distinctive difference between domesticated animals of several species (dogs, cats, cattle, horses, etc.) and their wild progenitors. Other morphological characteristics also arose in the same manner: some foxes had floppy ears until significantly older age than foxes from commercial population, some had rolled tails, and some had changes in the skull shape making them look more like dogs (Trut et al., 1991, 2009). It is important to note that multisystem changes were also observed in the population of foxes selected for aggressive behavior (Trut et al., 2017).

The multifaceted changes observed in the course of the selection of foxes for tame behavior affirm the destabilizing hypothesis of Dmitry Belyaev (Belyaev, 1979). The appearance of these new traits in a tame population could take place through several genetic mechanisms (Trut et al., 2021). Genes that were targeted by selection for behavior could have a pleiotropic effect and be involved in regulatory processes not only restricted to behavior, but that play an important role in animal development and physiology (Belyaev, 1979; Trut, 1999; Trut et al., 2009; Johnsson et al., 2021). Genetic hitchhiking could also lead to novel phenotypes, especially if genes involved in development or physiology are located on the same chromosomes and near genes involved in behavior. Selection for rare alleles of genes involved in development and physiology that happened to be on the same haplotype with selected alleles for behavioral genes could promote the development of novel phenotypes or increase the frequency of rare ones. In-phase alleles for both behavioral genes and neighboring genes on the same haplotype would be inherited together until sufficient generations passed for the haplotype to be broken by recombination events. Genetic drift is also plausible but taking into account that the higher rate of atypical phenotypes was observed during the early stages of selection for behavior (Trut et al., 2009) and the genetic structure of the fox populations (Kukekova et al., 2018), it is reasonable to argue that novel nonbehavioral phenotypes have appeared largely due to pleiotropy and hitchhiking.

The novel phenotypes observed in the fox tame population mimic behavioral, physiological, and morphological characteristics of domesticated animals (Trut et al., 2004, 2009, 2021). Some of these characteristics are so common across domesticated species that they are thought to be correlated and appear through the same biological mechanisms (Crockford, 2002; Wilkins et al., 2014). These common features of domesticated animals were described by Charles Darwin and recently titled the domestication syndrome (Darwin, 1868; Wilkins et al., 2014, 2021; Wilkins, 2017; Sánchez-Villagra et al., 2016; Wheat et al., 2019; Lord et al., 2020; Trut et al., 2020; Zeder, 2020). There are several hypotheses aimed to explain biological mechanisms underlaying phenotypes associated with domestication syndrome (Wilkins et al., 2014; Wilkins, 2020; Pendleton et al., 2018;

Johnsson et al., 2021). One of the widely used hypotheses states that neoteny is responsible for the retention of juvenile traits in domesticated species (Wayne, 1986). Although this hypothesis has not been rigorously tested using current scientific methods, it is important to notice that multiple behavioral and morphological phenotypes associated with delayed or truncated development have been observed in domesticated species as well as tame foxes (Coppinger and Coppinger, 2001; Trut, 1999, 2001; Trut et al., 2009). It was proposed that neotenic features of domesticated animals may be related to altered production of the thyroid hormone (Crockford, 2002) but this idea also remains to be investigated. A recent hypothesis that gained significant interest (Wilkins et al., 2014; Wilkins, 2020; Pendleton et al., 2018; Lord et al., 2020; Johnsson et al., 2021) is a neural crest cell hypothesis that states that altered migration of neural crest cells from the neural tube to final positions in the body can lead to a suite of domesticated phenotypes (Wilkins et al., 2014). The neural crest cell hypothesis received some empirical support in studies of dogs and tame foxes (Pendleton et al., 2018; Wang et al., 2018), but it was criticized in several theoretical publications (Lord et al., 2020; Johnsson et al., 2021). Until recently, it was almost impossible to study mechanisms of cooccurrence of behavioral and nonbehavioral traits in domesticated species, but newly available techniques, such as high-throughput sequencing, single-cell genomics, high-resolution imaging, and embryo editing (Simoes-Costa and Bronner, 2016), are expected to shed light on this question.

Genetics of fox behavior

The populations of tame and aggressive foxes provide a robust model for the identification of genes and gene pathways involved in behavior. Unlike modern dogs, the strain of tame foxes was created rapidly by selection focused solely on behavior and therefore holds advantages for genetic analysis. Although tame and aggressive fox populations have been carefully studied for several decades, only recently has it become possible to consider a systematic approach to identify the loci and molecular mechanisms controlling their behavioral differences. Classical genetic approaches to genome mapping had derived a rudimentary map of the fox genome by 1998, with a well-defined fox karyotype (Graphodatsky et al., 1995; Yang et al., 1999; Rubtsov, 1998; Graphodatsky and Radjably, 1981). To begin an attack on the molecular genetics of behavior in these foxes, however, we had to develop further resources including the fox genome (Kukekova et al., 2018; Rando et al., 2018), the fox genetic linkage map (Kukekova et al., 2007, 2012), an adequate set of appropriate molecular markers (Kukekova et al., 2004, 2012), a set of suitable pedigrees for mapping (Kukekova et al., 2011; Nelson et al., 2017), and a robust method for measuring behavior in resegregating pedigrees (Kukekova et al., 2007; 2011; Nelson et al., 2017).

To identify genetic regions implicated in fox behavior, we used two separate approaches: (1) genetic mapping of loci in experimental pedigrees; (2) identification of genomic regions differentiating the fox populations.

Genetic mapping of fox behavioral phenotypes

A program was instituted at ICG to crossbreed foxes from the tame and aggressive strains, producing an F1 population, and subsequently generating informative backcross and intercross pedigrees (F2). In the course of the selective breeding program, the behavioral phenotypes in the selected tame and aggressive populations quickly diverged, and separate scoring systems for the assignment of fox behavioral phenotypes were developed for the tame (Table 8.1) and the aggressive populations (Table 8.3) (reviewed in Trut, 1980a,b, 1999, 2001; Kukekova et al., 2005). However, a single, unified scoring system was required for measuring behavior in experimental pedigrees descended from crosses of the tame and aggressive strains. Individuals in such pedigrees have a wide range of behaviors and often exhibit fragmented or reshuffled elements of the behavioral patterns characteristic of the founder populations.

A new method was devised that could measure the variation in quantitative behavioral phenotypes between different fox populations as well as the resegregation of behaviors in experimental pedigrees. This new system was rooted in the traditional behavioral test used for selective breeding (Trut et al., 2004; Vasil'eva and Trut, 1990; Trut, 1980b, see Table 8.1). Fox responses to humans were evaluated in a standard series of four sequential steps that were videotaped (Kukekova et al., 2008, 2011):

- Step A observer stands calmly near the closed cage but does not deliberately try to attract the animal's attention;
- Step B observer opens the cage door, remains nearby but does not initiate any contact with the fox;
- Step C observer attempts to touch the fox;
- Step D observer closes the cage door, then stays calmly near the closed cage.

Because all foxes live under consistent conditions including similar interactions with humans and because their behavior is tested at precise time points using standard tests under constant conditions, the environmental factors that might influence behavior were held to a strict minimum.

An ethological survey of the videotaped tests identified over 300 discrete behavioral observations (traits), which could be reproducibly scored from video records in a binary fashion, for example, presence or absence. Examples, each simply scored Yes or No, include: "Wagging Tail?"; "Stays at the front wall of the cage?"; and "Ears pinned?" (Kukekova et al., 2008). Evaluation of these traits for informativeness, redundancy, reproducibility,

and consistency identified two overlapping sets of traits: (1) a minimal set of 50 traits that reliably distinguished fox populations along a tame—aggressive axis (Kukekova et al., 2008); and (2) a larger, 98 trait set (Kukekova et al., 2011) that includes all traits from the minimal trait set plus additional traits that capture other dimensions of fox behavior (Kukekova et al., 2011).

Fox video records were analyzed by scoring for the presence or absence of each of 98 binary traits and obtained scores were subjected to principal-components (PC) analysis to identify independent (i.e., uncorrelated) underlying factors (i.e., the principal components) that accounted for decreasing amounts of the total variance in observed behavior in the test (Kukekova et al., 2011; Nelson et al., 2017). In this analysis, the first two principal components, PC1 and PC2, accounted for 33% and 9% of the total behavioral variation, respectively (Kukekova et al., 2011). PC1 clearly distinguished tame foxes from aggressive foxes; F1 foxes yield intermediate values that extend into the ranges of both the tame and aggressive foxes. The scores of the backcross generations resegregate (Fig. 8.2A) such that mean values of PC1 in the different populations defined a linear gradient of heritable behavior, ranging from aggressive to tame, that clearly corresponds to the relative proportions of aggressive to tame ancestry in each population (Fig. 8.2A). PC2 did not follow a similar gradient (Fig. 8.2B). A review of the discrete behavioral traits that

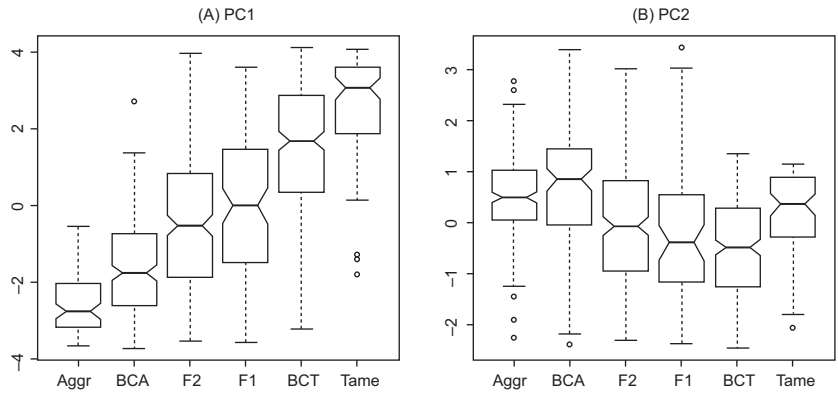


FIGURE 8.2 Population distributions for the first two principal components of silver fox behavior. (A) Distributions for principal component 1 (PC1); (B) Distributions for PC2. Aggr = "'aggressive"' founder population; BCA = backcross-to-aggressive; F2 = F2 population (F1 x F1); F1 = F1 population ("'tame"' × "'aggressive"'); BCT = backcross-to-tame population; Tame = "'tame"' founder population. Horizontal bars within each box indicate the population median. Confidence intervals for the medians are shown as notches such that two distributions with nonoverlapping notches are significantly different (at $P < 0.05$). The bottom and top edges of the boxes indicate the 25 and 75 percentiles. The whiskers indicate the range of data up to 1.5 times the interquartile range. Outliers are shown as individual circles. In each of the populations, the PC1 distribution pattern conforms to that expected for a heritable trait reflecting the proportional contributions from "'Tame"' and "'Aggressive"' ancestry. This is clearly not the case for PC2.

contribute to these two principal components demonstrated that PC1 and PC2 are comprised of very different aspects of behavior. PC1 comprises traits that distinguish overall aggressiveness from tameness, whereas most of the traits important to PC2 can be interpreted as distinguishing bold from shy behavior (independent of the degree to which a fox was tame or aggressive).

The analysis of fox video records also showed that in contrast to the behavior of foxes from tame and aggressive populations, the behavior of F2 foxes can be reshuffled among test steps, for example, some F2 foxes demonstrate tame behavior during step A but aggressive behavior during step C, therefore, we also performed PC analysis for each test step separately. This analysis yielded three PCs for each test step, where each PC explained at least 5% of behavioral variation during the step (Nelson et al., 2017). The PCs from individual test steps captured fox behavior in different contexts, for example, the highest and lowest values for PC1 at step A characterize foxes that are interested to interact with an experimenter versus foxes that avoid human contact, while extreme values for PC1 at step C differentiate among foxes enjoying tactile contact and foxes that show aggression when an experimenter tries to initiate a tactile contact (Nelson et al., 2017).

The quantitative trait loci mapping (QTL) of fox PC-defined behavioral phenotypes identified nine of the regions (loci) in the fox genome implicated in behavior (Kukekova et al., 2011; Nelson et al., 2017). The genomic regions identified for the PC1 phenotypes from the individual test steps did not overlap, suggesting that different loci regulate fox responses in different behavioral contexts. Three of the QTL were also identified as part of epistatic pairs of loci, suggesting a more complex genetic architecture of fox behavioral phenotypes than previously considered (Nelson et al., 2017).

Identification of genomic regions differentiating the fox populations

When next-generation sequencing technologies became available, the genome of a fox from our program was sequenced and assembled (Kukekova et al., 2018; Rando et al., 2018). We then resequenced genomes of 10 tame, 10 aggressive, and 10 conventional farm-bred foxes maintained in Novosibirsk and used the assembly as a reference to identify genomic regions with either significantly decreased heterozygosity in one of the three populations or increased divergence between the populations (Kukekova et al., 2018). In total, we identified 103 regions of interest, 30 of which overlapped with previously identified fox behavioral QTL (Kukekova et al., 2011; Nelson et al., 2017). Because the QTL intervals are broad and contain many genes applying a combination of approaches can facilitate the identification of the key genes located in identified genomic regions. In a proof of principle study, we analyzed one such region in detail and identified a strong positional candidate gene for tame behavior, the *SorCS1*, which encodes the main trafficking protein for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate

receptors and neurexins and suggests a role for synaptic plasticity in fox domestication.

The comparison of the fox genomic regions to the regions associated with domestication and positive selection in dogs (Vonholdt et al., 2010; Axelsson et al., 2013; Wang et al., 2013; Freedman et al., 2016) highlighted 45 significant overlaps, suggesting some common genomic responses to selection in the two species (Kukekova et al., 2018).

What the farm-fox experiment tells us about behavior

One of the most striking results of the farm-fox experiment is the clear demonstration of the influence of genes on behavior (Trut, 1980a,b, 2001; Kukekova et al., 2008, 2011a).

The farm-fox experiment showed that modification of a specific behavior (friendly response to humans) can affect other aspects of behavior as well. Tame foxes are eager to establish human contact from a very early age (Trut, 1999), develop a close attachment to their owners when raised in human homes (Trut, personal communication), and are as skillful as dog puppies in using human point-and-gaze gestures for finding the hidden food (Hare et al., 2005; Hare and Tomasello, 2005). Behavioral testing of kits from two farm-bred populations (i.e., the unselected population and the population selected for tame behavior), using the object-choice test, demonstrated that although fox communicative skills had not been used as a selection criterion (Trut et al., 2009), the tame foxes were as skillful in reading proximal point cues as age-matched puppies of domestic dogs and outperformed foxes from the unselected population (Hare et al., 2005). These results suggest that reduced fear and/or aggression to humans may be a prerequisite for the development of advanced interspecific communicative skills in canids. Thus the fox experiment provides an independent line of evidence for the argument that reducing fear and aggression can support the development of interactive behaviors.

Play activity of tame foxes persists into adulthood, and they actively seek communication with humans and other foxes (Belyaev et al., 1985; Plyusnina et al., 1991; Kukekova and Trut, 2010). Many other differences in behavior between tame and aggressive foxes, not directly related to their response to humans, remain to be investigated.

The selection of foxes for positive responses to humans led rapidly to the development of a behavioral repertoire with significant parallels to that of domestic dogs. The prehistoric domestication of the dog from the wolf may have taken a long time and more generations, but in all probability was similarly achieved through selection for behavioral modifications. The similarities between the behaviors of domesticated foxes and dogs suggest that the behavioral response to humans in these two species could involve similar sets of genes.

Summary

Archeological and molecular data have well documented the long history of the coexistence of humans and dogs. Although the full scenario of historical dog domestication remains to be determined, the behavioral differences between dogs and wolves strongly argue that selection for behavior was the force that created "Man's best friend." The fox domestication experiment demonstrated that many behavioral characteristics that differentiate dogs from wolves can be obtained by selection solely for a friendly response to humans. The rapid progress in the selection of foxes for tame behavior strongly suggests that selection was acting on genetic variation preexisting in the founder fox population. These results support dog domestication hypotheses that suggest that predog wolves underwent natural selection for behavioral traits that allowed them to coexist with humans. Identification of loci that both influence tame behavior in foxes and are homologous to regions in the dog genome supporting genetic signals related to the selection of dogs from wolves leads to the intriguing hypothesis that domesticated behavior in dogs and foxes may have a similar genetic basis.

Not only do dogs differ in their behavior from wolves, but different breeds of dogs are also characterized by genetically determined differences in behavior. Although the latter differences are highly characteristic and readily recognized, each such behavioral pattern likely represents a synthesis of many discrete behaviors. This synthesis has been clearly demonstrated in the fox study. For example, the apparently simple response to human presence can be regulated by different loci in different contexts (Nelson et al., 2017). A detailed understanding of the genetic basis of behavior in dogs requires well-defined phenotypes, as well as control of environmental factors and gene-environment interactions that can strongly influence behavioral outcomes. These are not as easily implemented in dog research as in the fox study.

Although domestication has for many years been of great interest to scientists, most notably Darwin, until recently basic questions concerning the genetic mechanisms involved have been difficult to address. With modern advances in molecular genetic technologies, however, it is now feasible to pursue research on diverse organisms to find links between genes, brain function, and a wide range of social behaviors (Robinson et al., 2005, 2008; Hecht et al., 2019, 2021).

We expect that identification of the molecular mechanisms underlying domesticated behavior in foxes and dogs will help us to understand basic biological principles guiding social behavior in mammals and to provide insights into our own behavior as well.

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Chapter 9

Behavioral genetics in pigs and relations to welfare

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ABSTRACT
Research in behavioral genetics is important for pig welfare. Consequences of the ongoing selection for high production on pigs' behavior need to be studied, as well as possibilities to select directly for behavioral traits. The Farm Animal Welfare Council's definition of welfare is based on five freedoms related to hunger and thirst, discomfort, pain, injury or disease, fear and distress, and normal behavior. All these freedoms are associated with pig behavior. Pig breeding programs could be further developed by including behavioral traits relevant for welfare.

Introduction

The human global population is growing, and there is an increased demand for pork. At the same time, the competition for agricultural land is increasing and the negative environmental influence of pig production must be decreased. Thus, there is a need for increased efficiency at all levels of the production chain, starting with the pig's genotype. But intense selection for production traits such as feed efficiency may decrease welfare, for example, by decreasing the capacity to adapt to stress. It is a challenge to increase efficiency without decreasing pig welfare.

The domestication of the pig started approximately 10,000 years ago. Since then, the animal has developed from a small wild boar sow giving birth to a litter of six slow-growing fat pigs once a year to today's large sow, which produces 25 fast-growing lean pigs a year. During the long process of domestication, pigs' behavior has changed genetically. Fear of humans has decreased, and the pigs have become much easier to handle. More recently, the genetic progress in production traits has been enormous. However, breeding may threaten animal welfare due to undesirable correlated effects in behavior, metabolism, reproduction,

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and health traits. Fortunately, the genetic tools that have been used to increase production can also be used to decrease negative side effects of selection and improve traits important for welfare.

Recent developments in recording techniques and in genomics will facilitate selection for behavioral traits important for animal welfare. Genomic breeding values estimated based on a combination of genomic markers and phenotype records have higher accuracies than traditional breeding values, which is especially valuable for traits that are complicated to record. With sensors keeping track of individual animals and image-based recording methods, aggressive behavior can be recorded.

Pig behavior

Explorer and generalist

Pigs have extensive social skills and relatively low sensitivity to confinement; traits that favored domestication. After generations of successful genetic selection to increase pigs' performance, modern pigs differ from their wild counterparts not only in production and reproduction but also in behavior. The relaxation of natural selection has reduced the degree of fear of predators and made pigs easier to handle. It is, however, important to remember that only the frequency and the threshold at which behaviors are triggered have changed. No new behavior has appeared and none has disappeared.

Pigs are sentient animals. They express strong behavioral needs relating back to the behavior of the wild boar in nature. Welfare problems arise if those needs are not met. When the production system does not permit a sow to build a nest for farrowing (because there is no building material) it may redirect the behavior into bar-biting, which may become a behavioral disorder (Lawrence and Terlouw, 1993). The behavior is a good indicator of welfare because it refers to strong decision-making and motivational processes in the pig (Dawkins, 2004; Kittawornrat and Zimmerman, 2011).

Pigs are probably the "smartest" animals among livestock (Broom et al., 2009). They can make contextual associations and memorize them for a long time. Following the example of wild boars that have their home range in memory (Spitz, 1986), de Jonge et al. (2008) showed that piglets were capable of associating music to access to a playroom, and suggested that music can elicit play behavior. Pigs' hearing range is similar to that of humans and they show aversion to loud noise. From birth, piglets rely on olfactory cues from the dam and its udder. In adulthood, recognition between individuals also depends on olfactory cues (Curtis et al., 2001). With its highly developed senses and its large cognitive abilities, the pig has a very good perception of its surroundings.

Being a generalist, the pig is a forager that can eat almost anything. Domestic pigs fed from an automatic feeder eat around seven times a day

and spend almost 1 hour feeding per day during the growing–finishing phase (von Felde et al., 1996). On pasture, feed-related behaviors such as rooting, grazing, and exploring substrate account for 75% of daily activity (Stolba and Wood-Gush, 1989). The explorative behavior is promoted by pigs' capacity to detect odors that they memorize for several hours (Signoret et al., 1975). In parallel, pigs show a high level of curiosity. Given the choice, they choose to enter environments that contain new objects to investigate, and this investigation is not only related to feeding motivation (Wood-Gush and Vestergaard, 1991); the investigation of novelties is rewarding in itself.

Adaptation and learning

The pig is a generalist that can adapt to many different environments (Fig. 9.1). When domestic pigs are released into the wild, they show a large capacity for behavioral adaptation. Feral pigs have colonized many different types of habitats, for example, in Australia (Edwards et al., 2004) and in the United States (Wyckoff et al., 2009). Dzieciolowski et al. (1992) report that under the pressure of natural selection, the characteristics of feral pigs return to those of wild boars.

Two common tests used for genetic studies of individual temperament are the open-field test and the novelty test. The open-field test was developed for rodents. Animals are tested in an open field to induce a conflict between aversion and voluntary exploration of a novel environment. Locomotion and



FIGURE 9.1 The pig is a generalist. Wild boar pigs thrive in the city center, as these pigs living in Haifa. *Photograph: Abir Sultan / EPA / TT.*

exploration are used as indicators of adaptation to change. A short period to the first move in the open field and a high level of activity may reflect a low level of anxiety (Réale et al., 2007), but the interpretation of pigs' reactions has been questioned as pigs and rodents do not exhibit the same motivational processes. The response to novelty can be measured by introducing a novel object in the home pen or in a test arena after a period of acclimatization to the novel environment. Usually, the first reaction to the novel object is to freeze. The latency until the animal reacts, that is, moves or gets in contact with the object, is used as a measure of its boldness (Réale et al., 2007).

Even though pigs show the ability to modify their behavior to adapt to the present environment, many behaviors are repeatable across situations and some of them are inherited. Behavior traits do, however, differ from many other traits in that they are changed by experience and learning. For instance, there is a strong effect of age on the interest in a given object used in the novelty test (Docking et al., 2008). Early contact with humans influences subsequent levels of fear (Hemsworth and Barnett, 1992). Thus individuals in a group of young pigs may enter the growing–finishing facility with very different experiences. They may also have different genotypes, for example, regarding explorative and aggressive behavior.

The social pig

The group size is quite similar for wild boars, feral pigs, and farmed pigs, although these animals live in a wide range of habitat types and resource availability. Under natural conditions, the groups are based on matriarchal hierarchies (Stolba and Wood-Gush, 1989), and males are loosely associated with these groups (Mauget, 1981). On the farm, pigs born in different families are often grouped together in small pens. Whereas aggression is avoided in the wild, where different groups seldom meet, mixing with unacquainted pigs is frequent in pig production. When unacquainted pigs meet, they fight to establish dominance. During the first 24 hours after mixing, most pigs are involved in many fights that lead to energy expenditure and injuries such as skin lesions. Turner et al. (2006a) counted skin lesions on pigs after mixing as a measurement of aggressive behavior. Ten percent of the pigs had more than 50 skin lesions. The frequency and intensity of aggressive interactions decline over time after mixing, until social relationships stabilize. However, an ongoing lower level of aggression persists to maintain social relationships.

Aggressiveness in pigs is known to be repeatable over time and across different situations, and it is partly influenced by the genotype. A pig's decision to engage in an aggressive interaction or not may be made according to the relative costs and benefits of the behavior, which will vary depending on resource scarcity and the other pigs' behavior (Enquist and Leimar, 1983). Arey and Franklin (1995) studied groups of 15 newly mixed pigs and found that in 60% of the dyads (pairs) there were no fights.

Pigs can identify unfamiliar individuals in large groups of up to 80 pigs (Turner et al., 2001). Gilts and sows can remember their group mates and identify them when they meet again after several weeks (Arey, 1999). Thus fighting can be avoided. The stability of the group of pregnant sows is maintained by a dominance hierarchy that depends on subordinates avoiding the dominant sows (Jensen, 1982). The dominance order is, however, resource relative; group members may have different dominance orders for different resources (Lindberg, 2001). Whether it is worth it or not to initiate a fight depends on group size and the predictability of the resource, for example, feeding. In large groups, a higher number of competitors dilutes the effectiveness of aggression and increases its energetic cost (Fraser et al., 1995).

Individual differences in aggressiveness can be measured in a resident−intruder test (Réale et al., 2007). The tested pig, that is, the resident, encounters in its home pen an intruder (that should be of slightly smaller size), and the latency until an attack by the resident is recorded. Recording aggressive behavior among pigs in a group is difficult as the pigs must be identified individually. Aggressiveness can be investigated by direct or video observations, either by recording the total number of initiated and received attacks for each pig or by recording the identity and outcome of each dyadic encounter.

In general, pigs search for positive and close interactions with humans. Fear of humans is an indicator of low welfare, and different methods are used to record fear. One way, used by Velie et al. (2009) and others, is to let a person unfamiliar to the pigs enter the pen and stand there motionless, and the latency for pigs to approach and touch the human is recorded. The success of this test relies on the pigs' motivation to voluntarily approach human. The trait "easy to handle" can be measured during routine work, for example, when pigs are moved between pens or weighed.

Tail biting

Pigs' behavioral response to chronic stressors in the environment may translate into abnormal behavior such as tail biting. It could be a redirected behavior related to the need to explore and forage. Even so, tail biting can also be observed in outdoor production (Walker and Bilkei, 2006). Pigs performing tail biting with a high frequency also perform much ear-biting and belly-nosing. Brunberg et al. (2011) identified three types of pigs: biters, victims, and neutral pigs that never perform nor receive tail biting even during an ongoing outbreak. The frequency of biters and the severity of tail biting are highly influenced by the environment. The number of injured tails is easy to record, but to identify the biters is quite complicated. Breuer et al. (2003) used pigs' motivation to chew a rope as an indirect measure of their biting tendency.

Sexual behavior

In most countries, males raised for slaughter are castrated, but there are exceptions like the United Kingdom. Due to welfare reasons, the European Union wanted to ban surgical castration from 2018. This did not happen, but the European Union continued to promote the rearing of entire males (European Commission, 2019). Entire males are more likely to show aggressive behavior and are more active than castrates (Cronin et al., 2003). They also display more sexual behavior such as mounting (Rydhmer et al., 2010). In boars used for mating and semen collection, there is genetic variation in libido traits, with heritabilities around 0.15 (Rothschild and Bidanel, 1998).

By means of pheromonal communication, gilts and sows grouped together synchronize estrus. During estrus, the female shows standing reflex and there is a genetic variation in the ability to show this sexual behavior. The heritability of this categorical trait was estimated at 0.3 on the underlying scale in a research herd (Rydhmer et al., 1994). Recording of estrus symptoms was later performed in Norwegian nucleus herds, but almost no genetic variance was found in that environment (Holm, 2004).

Maternal behavior

The pig is an exception among the ungulates regarding prolificacy; it has more in common with rodents and many carnivores. The sow produces a very large litter for being such a large animal. At the approach of farrowing, the pregnant sow becomes intolerant of group mates (Stolba and Wood-Gush, 1989). In the wild, the sow leaves the group 3 days before farrowing and searches for a suitable site where she builds a nest with materials from the vegetation. In the farrowing stable, the sow also builds a nest if given building material and space. Sows spend around 15 hours on nest-building activities (Thodberg et al., 1999) and in the absence of material, sows direct their activity at the floor, walls, and bars (Wischner et al., 2009). They humidify the floor with their snout and paw insistently with their front legs. The nest-building activities are very similar in wild boar sows and domestic sows housed indoors (Stolba and Wood-Gush, 1989).

When the first piglet is born, the sow reduces the number of postural changes and lies still until most piglets are born. The farrowing takes on average 4 hours (Wallenbeck et al., 2009). Sows differ in their maternal skills, which influence the probability of survival of the piglets. Before lying down, some sows carefully check if there are any piglets, to avoid crushing them. Others do what Wechsler and Hegglin (1997) describe as "flopping straight down," which is a great risk for the piglets. An attentive sow reacts to piglet screams by changing posture, and thus many piglets are saved. Sows' reactions to the sound of screaming piglets have been used as a measurement of maternal behavior (Grandinson et al., 2003). Unlike the ewe, the

sow does not lick her young. Instead, the piglets initiate frequent nose-to-nose contact with their mother. Most sows can bond to alien piglets, which is convenient in high-producing herds as it allows cross-fostering as a means to optimize piglet survival.

Nursing is based on a sophisticated interaction between the sow and its piglets (Canario, 2006). After vigorous competition among littermates during the first days, each piglet gets its own teat. The sow nurses the piglets every hour, day and night. She initiates the nursing event by grunting and lying down on her side. The piglets respond by massaging the udder for a few minutes, which stimulates oxytocin release resulting in the milk let-down. After the milk ejection, which is around 20 seconds, the piglets continue to massage the udder until the sow interrupts nursing by standing up or rolling on her belly, or until the piglets leave the udder or fall asleep. The total nursing event takes on average 5 minutes but the variation is large (Wallenbeck et al., 2008).

Farm Animal Welfare Council's five freedoms

The Farm Animal Welfare Council's definition of animal welfare (FAWC, 1992) lists five freedoms. These freedoms define ideal states and form a comprehensive framework for analysis and discussions of animal welfare within the proper constraints of an effective pig production unit. The five freedoms are as follows:

- Freedom from hunger and thirst—by ready access to fresh water and a diet to maintain full health and vigor.
- Freedom from discomfort—by providing an appropriate environment including shelter and a comfortable resting area.
- Freedom from pain, injury, or disease—by prevention or rapid diagnosis and treatment.
- Freedom from fear and distress—by ensuring conditions and treatment that avoid mental suffering.
- Freedom to express normal behavior—by providing sufficient space, proper facilities, and the company of the animal's own kind.

Although the five freedoms stated by FAWC do not refer directly to genetics and breeding, we use them as a structure for this chapter.

Freedom from hunger and thirst

Are hunger and thirst welfare problems for pigs? In many countries, young pigs during the growing–finishing phase, as well as lactating sows, are given *ad libitum* access to feed and water. There is sometimes competition between pen mates at the feeder or water nipple. Such competition is, however,

probably more related to "fear and distress" or "discomfort" than to "hunger and thirst".

Some newborn piglets do, in fact, starve, and most piglets have a restricted access to milk. A piglet ingests around 40 g of milk per meal, and the piglet's total milk intake can be estimated as four times the increase in body weight (Noblet and Etienne, 1989). Piglets have a very high capacity to eat (or rather to drink), and it is not known whether the difference between milk intake capacity and realized milk intake is related to hunger, but piglets starving to death are of course a welfare issue. Piglet mortality and growth are governed by both the genes of the sow and the genes of the piglet (Grandinson, 2003). Sows' milk production (recorded as litter growth) also has a genetic background (Lundgren, 2011).

A lactating sow producing as much as 15 L of milk per day cannot eat enough to fulfill its nutritional needs during lactation. One way to overcome this restriction and increase milk production could be to select for increased appetite or voluntary feed intake during lactation. Pig breeders seldom think of appetite as a behavioral trait, but humans' food intake is often discussed in relation to behavior. Selection for increased voluntary feed intake of lactating sows was proposed by Eissen et al. (2000). Problems with low appetite increase with increasing temperature. Bergsma and Hermesch (2012) have shown that breeding for reduced thermal sensitivity of feed intake is possible. Lundgren et al. (2013) found genetic correlations between sow appetite and piglet growth rate (higher appetite—heavier piglets) and between appetite and body condition at weaning (higher appetite—better body condition). Selection for high voluntary feed intake may thus improve the welfare of both piglets and lactating sows, but what would the consequences of selection for a larger appetite in lactating sows be for the welfare of dry sows?

Dry sows never have ad libitum access to feed and the high-energy, grain-based feed used for sows is quickly digested and results in long-term periods of hunger (EFSA, 2007). One way of assessing total welfare impact is to take the number of animals, severity, and duration into account, and sows are lactating for less than a quarter of the year. The genetic correlation between appetite during different phases of the sow's life is not known, but it seems likely that selection for high voluntary feed intake of lactating sows will result in hungrier dry sows. The goal conflict between high voluntary feed intake during lactation and restricted feed provision during pregnancy could be solved by giving free access to roughage or straw in addition to selection for high appetite. Straw not only reduces hunger; it also provides an occupation. Feed restriction and a boring environment may result in stereotypies. Spoolder et al. (1995) showed that straw reduces the development of excessive bar manipulation in sows on restricted feeding. It has been proposed that tail biting (as well as feather pecking) is a redirected foraging behavior (Brunberg et al., 2016) and it can be assumed that straw and other edible rooting materials improve the welfare of all pigs.

There are genetic differences in eating behavior among young, growing pigs. When fed with an automatic feeding station, the size and duration of each meal, distribution of meals over time, etc. can be recorded (Fig. 9.2). The duration of meals and feeding frequency are heritable traits ($h^2 = 0.4-0.5$, Labroue et al., 1997). Fernández et al. (2011) found specific feeding strategies for different breeds. The number of meals per day was negatively correlated with the sizes of meals and duration of meals. Large White pigs were "nibblers and fast eaters," Pietrain pigs were "nibblers and slow eaters," Duroc pigs were "meal and slow eaters," and Landrace pigs were "meal and fast eaters". According to Fernández et al. (2011), the "meal and fast eater" strategy is related to a higher growth rate. Several genes or markers with a significant effect on feeding behavior have been found. The MC4R gene, which codes for a melanocortin receptor, has a positive effect on daily feed intake, probably mediated through the central control of appetite (Kim et al., 2000). Zhang et al. (2009) identified a quantitative trait loci (QTL) on chromosome 7 for the number of visits of pigs to the feeder.

Although pigs are gregarious animals, aggressive interactions often occur during feeding (Rydhmer et al., 2006). In a study by Jonsson and Jørgensen (1989), where feed was a restricted resource, social rank was correlated to the growth rate (high rank—high growth rate). The growth rate is included



FIGURE 9.2 Pigs often eat together, but when individual feed intake should be recorded, they have to eat one by one in an automatic feeding station. *Photograph: Nils Lundeheim.*

in the genetic evaluation of all pigs, and in many breeding programs, young pigs are tested in systems with automatic feeders where only one pig at a time can eat. The genetic correlation between feeding behavior in this system and feeding behavior when the pigs are group fed in a trough is not known. Analysis of high-throughput data from automatic feeders have shown that Landrace pigs make significantly fewer visits per day than Duroc and Yorkshire pigs (Do et al., 2013; Cross et al., 2000), and feed intake rate is higher in Landrace and Yorkshire pigs than in Duroc (Do et al., 2013; Xin et al., 2016). In Finnish finishing pigs, Kavlak et al. (2021) estimated the heritability for number of visits per day at 0.30, and 0.28 for time spent in feeding per visit, and 0.14 for time spent in feeding per day. Vargovic et al. (2021) studied the eating behavior of dry sows and found a genetic variation in feeding behavior. They conclude that time spent eating and the prevalence of missed or low intake meals can be relevant selection traits and suggest that slower eating sows can be stressed and reduce their feed intake in unprotected feeding systems.

Residual feed intake (RFI) is a measure of feed efficiency that accounts for the animal's energy requirements for production and maintenance. It is calculated as the difference between observed and predicted feed intake. The RFI concept was proposed as a selection trait for several species in the 1990s. With today's increasing awareness of the climate impact of animal production, breeders' interest in RFI has increased. Low RFI in the growing pig is genetically correlated to high leanness and low feed intake. Selection for decreased RFI could be controversial from a welfare standpoint, given that the genetic variation in RFI partly reflects the genetic variation in activity and potentially also stress response (Luiting and Urff, 1991). RFI indirectly measures the quantity of buffer resources available for activity, immune system, some metabolic processes, and stress response. According to the resource allocation theory, low RFI may thus reduce the ability to cope with stressors as the animals' buffer for stress response is reduced. This theory has not been confirmed in studies of pigs from a large French experiment with divergent selection for RFI (Gilbert et al., 2017). In another study, pigs selected for low RFI were calmer in the home pen and less reactive to novelty (Colpoys et al., 2014)

Sadler et al. (2011) found a positive genetic correlation between activity and RFI (low RFI—low activity level), and a similar correlation was also found in the French selection experiment (Gilbert et al., 2017). Reduction of RFI by selection increases feed efficiency at the expense of reduced feeding activity, owing to a positive genetic correlation between RFI and feeding duration (von Felde et al., 1996). Young et al. (2011) compared a line selected for reduced RFI with a control line in pens where pigs from both lines were mixed and fed with a single-space electronic feeder from 3 months until slaughter. Pigs selected for low RFI ate less meals per day than control pigs, especially during peak eating times. The authors conclude

that feed efficiency may be affected by feeding behavior because selection for decreased RFI results in pigs that spend less time eating and eat faster.

Freedom from discomfort

According to FAWC (1992), animal welfare includes "Freedom from discomfort—by providing an appropriate environment including shelter and a comfortable resting area". What is an appropriate environment for a pig, and could the environment be more or less appropriate depending on the pig's genotype? In FAWC's report on the welfare of pigs kept outdoors (FAWC, 1996), it was written: "Breeding companies, and those responsible for the selection of breeding stock to be kept on outdoor enterprises, must ensure that only those strains of pigs with the genetic potential to thrive in the conditions provided are used". Thus FAWC assumes that there are important genotype-by-environment interactions (G × E) related to welfare. Maternal behavior is often mentioned as a trait of extra importance outdoors (Wallenbeck, 2009). Another example could be genetic differences in coat color, where pigs with alleles for white color probably have a higher risk of getting burnt by the sun if there is no shade. In the tropics, cattle breed differences have been observed with regard to seeking shadow (Hernández et al., 2002). Cinta Senese (CS) is an autochthonous pig breed used in pasture-based production in Italy. CS pigs were compared to Large White crosses (LW × CS) kept in the same paddocks for 11 months (Parrini et al., 2019). In general, CS pigs spent less time on grazing than LW × CS pigs. The time spent on grazing was reduced in both breed groups during the hot summer month of July, but the reduction was larger for CS than for LW × CS pigs.

The variation in temperature is larger outdoors than indoors, and both very cold and very hot weather can lead to thermal stress. High temperature is a welfare problem also indoors in many countries, during summer in tempered areas, and all year round in tropical areas. Furthermore, heatwave incidence and duration will increase with the ongoing climate changes. To maintain normal metabolism, animals' core body temperature needs to remain relatively constant. Pigs are especially affected because they cannot dissipate heat by sweating (Renaudeau et al., 2011), resulting in heat stress. Heat stress occurs when the pig is exposed to at least two consecutive days with a maximum temperature higher than 28°C (Zumbach et al., 2008) and it has a negative impact on animal welfare causing physiological changes and increasing disease incidence. Activity increases heat production and pigs spend more time lying and less time eating as temperature increases (Brown-Brandl et al., 2001).

Selection for increased lean growth has led to higher total heat production in modern pigs, which increases the pressure to maintain homeothermy when it is hot. There are breed differences in heat stress susceptibility and

Gourdine et al. (2006) found signs of G × E when comparing Creole and Large White sows, and genetic variation in heat stress tolerance in lactating Large White sows in a tropical climate (Gourdine et al., 2017). Thus current breeding goals can worsen heat stress as high-producing animals produce more heat, but animals can be selected for increased heat stress tolerance. Genetic evaluations for heat tolerance are often based on the reduction in performance recorded under heat waves, as compared to temperate periods (Carabaño et al., 2019).

Cross et al. (2018) compared feeding behavior of growing pigs between days classified according to temperature humidity index as Normal (<23°C), Alert, Danger, and Emergency (≥29°C). Feeding behavior was measured as number of times per day at the feeder (recorded every 20 seconds). The heritability for changed feeding behavior between Normal and Alert or Emergency days was estimated at 0.1 and between Normal and Danger at 0.2. Several significant associations were found in a genome-wide association study of the changed feeding behavior. Genes involved in immune response and function were overrepresented in regions associated with changed feeding behavior. The authors conclude that "candidate genes identified in this work, including heat shock proteins and stress response, merit further investigation and may facilitate genetic selection for improved grow-finish performance during heat stress events".

Kanis et al. (2004) used thermal discomfort as a model trait when discussing the ability to breed pigs for increased robustness and thereby increased welfare. Based on the physiological and behavioral responses of pigs to changing temperature, different welfare zones can be identified and the individual transition points between these zones can serve as a selection trait, aiming for a broader welfare zone. An alternative is to test pigs under harsh conditions and select those animals with the least problems associated with coping (Kanis et al., 2004), but ethical evaluations should be done before performing such tests.

In intensive, large-scale indoor production systems, a varying environment is probably not the main welfare problem. On the contrary, the uniformity and lack of events may be a large challenge for growing pigs. Thus we must stress the question "Appropriate environment for whom?" Are there any lines of pigs with the genetic ability to "thrive" in the ordinary indoor conditions where pigs are usually kept during the growing–finishing period? The pig is an explorative animal, but there is not much to explore in pens for growing pigs (see Figure 9.2 for an example). Breed differences in explorative behavior have been found; in a comparison between young Large White, Landrace, and Duroc pigs, the Durocs were more exploratory (Breuer et al., 2003). Meishan sows perform more explorative behavior than Large White sows (Canario et al., 2009). The members of the Federation of Veterinarians of Europe expressed concerns about breeding resulting in "animals with unnatural... behavioral characteristics" (FVE, 1999). The meaning

of natural and unnatural for domestic animals will be discussed later, but uncurious pigs could be regarded as unnatural.

"Freedom from Discomfort" also implies providing "a comfortable resting area". No matter how well designed the pen or the floor is, there will be little rest if the pigs are continuously disturbing each other. Entire males perform more social behavior than castrates and too much social behavior seems to decrease animal welfare even when it is not painful (Rydhmer et al., 2010). Nosing, pushing, sniffing, and nibbling are social behaviors normally performed by pigs. Bench and Gonyou (2007) found that breed and also sire within breed had a significant effect on belly-nosing and sucking behaviors in young, weaned pigs. In a breed comparison by Breuer et al. (2003), Duroc pigs performed less belly-nosing than Landrace and Large White, suggesting a genetic background to these behaviors. If rearing of entire males for slaughter would become the dominating production system, selection for an optimal frequency of both aggressive and nonaggressive social behaviors could be relevant.

Freedom from pain, injury, or disease

Locomotion and leg problems

Pigs suffering from pain can be identified by recording and analyzing pigs' movements and activity patterns. In sows, unusual changes in activity can be related to adaptation problems when moved to the farrowing unit, or to diseases or leg problems. On the other hand, taking a long time to move from standing to lying may be an indicator of the sow carefully avoiding to crush piglets. Thus movement records must be interpreted with care. The study of sow behavior and health over longer time periods is a fairly recent approach that will enable to define time windows when sow behavior is highly related to piglet mortality (Canario, 2020; Girardie et al., 2022). Sows' postural activity has a large genetic variance that can be exploited in selection to reduce piglet losses indirectly, for example, by choosing sows that give easy access to the udder to their piglets in early lactation and that are careful when they change posture. Sows with leg problems often show a sudden and uncontrolled lying behavior that increases the risk of piglet crushing. Leg weakness is a common cause of culling, but difficult to detected in confined sows during lactation. It can be assumed that sow postural activity is an overall indicator of both maternal behavior and health status of sows, both of which have a strong influence on piglet survival.

Monitoring animals over a long time period enables to detect changes in activity level. With visual observations (direct or by video), long time periods for a large number of animals are not feasible in practice. With the fast evolution of sensor technologies, we can expect an automatic collection of large amounts of various behavioral data. Computer vision and accelerometers can

be used to analyze sow behavioral changes over time. Accelerometers have been widely tested to record pig behavior (e.g., Ringgenberg et al., 2010; Oczak et al., 2016; Matheson et al., 2017). They are generally cheap, and data acquisition and analysis are relatively straightforward. However, the quality of the data depends on the sensor's position on the animal, such as neck, leg, back, or ear. Embedding the sensors on the animals is the main limitation to their general use.

Information from accelerometers fixed on the back of sows gave a first insight into the possibilities of selecting for sow postural behavior as a means to limit the crushing of piglets (Matheson et al., 2017). The heritability for the accelerometer trait "pitch change" was estimated at 0.32 ($\pm$ 0.17) but the heritability for "roll change" was much lower (0.03 ± 0.11). Both traits were genetically correlated to piglet crushing. It was concluded that piglet survival can be addressed by incorporating sow-activity traits in breeding programs. However, the authors admitted that the correlations between sow conformation and accelerometer traits merit a more detailed investigation before new selection traits can be implemented. Cameras raise increasing interest because they can be used to monitor several features, such as posture, live weight, and water intake patterns. For example, sow postural activity in a crate can be recorded with a very good accuracy (Bonneau et al., 2021) and the data can be analyzed with convolutional neural networks. We foresee that this combination of recording and analyzing locomotion data will result in new phenotypes associated with welfare in a near future.

Tail biting

Pigs are omnivorous and highly explorative. In barren production environments, this motivation to explore can be expressed as tail biting and other oral behavior directed toward pen mates. A parallel is seen in poultry (also an omnivorous farm animal) where feather pecking is a severe welfare problem (Brunberg et al., 2016). The barren environment is, however, not the only cause of these damaging behaviors; tail biting is difficult to prevent because of its multifactorial origin. The most extreme preventive treatment is to cut off the tails as a means of improving pig welfare. Routine tail docking has been prohibited for many years within the European Union (EU Directive 91/630 EEC). The EU law states that "neither tail docking nor reduction of corner teeth must be carried out routinely but only where there is evidence that injuries to sows' teats or to other pigs' ears or tails have occurred". In 2017, 77% (median = 95%) of the pigs in the EU were still routinely tail-docked. Sweden and some other EU countries have banned tail docking since many years. In Sweden, tail biting or other tail damage is observed in 7% of the pigs at the slaughter plant (Keeling et al., 2012). Is selection against tail biting possible, as a complement to environmental improvements? According to the Code of good practice for farm animal

breeding and reproduction organizations (CODEEFABAR, 2020a), breeding organizations improve animal welfare by "breeding animals in such a way that welfare unfriendly practices could be avoided or minimized" and tail docking is given as an example of such a practice.

Tail biting is a difficult trait to study because it is hard to identify the biter. Breuer et al. (2005) defined biters as pigs involved in more than 50% of the tail biting incidences within 10 minutes of observation. Tail biting is not performed by all pigs, almost 90% are never observed to perform tail bites (Brunberg et al., 2011). With advances in phenotyping with new technologies, large-scale recording of victims and performers may become possible, as discussed by Canario et al. (2020).

Breed differences in the tendency to tail-bite group mates have been found by Breuer et al. (2003). Nordic studies show that Landrace and Duroc pigs tail-bite more often than Yorkshire and Hampshire pigs. Yorkshire pigs are tail-bitten more often whereas Hampshire pigs are tail-bitten less often than the other breeds (Sinisalo et al., 2012). However, Lund and Simonsen (2000) found no clear breed effect. The genetic influence on this behavior can be more relevant within than between breeds.

Around 3% of the pigs in the study of Breuer et al. (2005) were classified as biters. The heritability of this "yes or no trait" was estimated at 0.3 (after transformation to an underlying continuous scale) in Landrace pigs. The corresponding heritability was zero in Large White pigs. At the genetic level, tail biting is unfavorably correlated with growth rate and leanness. A genome-wide association study was done on records of tail biting from Norwegian farms. Different chromosomal regions on the pig genome were associated with the delivery and receipt of tail biting behaviors (Wilson et al., 2012).

Initial studies in finishing pigs showed no genotype-by-environment interactions ($G \times E$) for exploration, and aggressive and nonaggressive biting (Hill et al., 1998; Guy et al., 2002), but a more recent study highlighted strong $G \times E$ for the receipt of tail bites (Canario and Flatres-Grall, 2018). Indications of $G \times E$ for these behaviors mean that conclusions from genetic studies must be drawn with care. Indeed, given that variability in behavior is influenced by environmental factors, a genotype can be used for production in an environment for which it is not adapted, and as a consequence, the effectiveness of selection can be reduced.

Measuring traits related to biting remains challenging. Injured animals can be identified by repeated visual monitoring, a method that is highly time-consuming and only provides estimates of infrequent, sporadic, or unpredictable behaviors. Lesions on the tail, ears, vulva, or skin identify the victim but not the biter, and it is the latter that is of particular interest in breeding against the behavior. Technological advances in the field of automated recording will be crucial in identifying attacks, based on the concomitant tracking of animals and the identification of harmful social interactions (Liu et al., 2020; D'Eath et al., 2018). By developing an aggression

identification algorithm based on the accelerations between two adjacent video images, Chen et al. (2017) were able to distinguish between medium- and high-speed aggressions with an accuracy greater than 95% for each of the two traits.

To get a better understanding of the molecular mechanisms of tail biting, Emma Brunberg studied gene expression in the brain (hypothalamus and prefrontal cortex) in her Ph.D. project (Brunberg, 2011). She used pigs from a Finnish herd and analyzed the abundance of RNA molecules from specific genes. There were differences in gene expression between biters and victims and between these types and neutral pigs. Neutral pigs are pigs not involved in tail biting although they are housed together with biters. Many of the genes differed between neutral pigs and the other categories. The EGF gene that codes for an epidermal growth factor and has an important function in the dopaminergic system was less expressed in neutral pigs. This gene is involved in novelty-seeking in humans (Keltikangas-Järvinen et al., 2006). Furthermore, the GTF2I gene that is involved in sociability was upregulated in the hypothalamus of neutral pigs. Also, the GHRL gene that codes for a hormone related to appetite and the PDK4 gene related to fat content were upregulated in the neutral pigs. More recently, a genome-wide association study of metabolites showed that pigs with a high risk of becoming victims may have impaired lipolysis process (Dervishi et al. (2019). The results give further grounds to the suspicion that selection for high production is followed by some abnormal behaviors directed toward group mates.

The influence of the gut microbiota on humans' and animals' behavior is a growing research area. The genotype of the host seems to influence the establishment of its microbiota (Goodrich et al., 2014). Gut microbiota can act on the adrenal cortex and via neurotransmitters on the brain, thereby predisposing the host to develop abnormal behavior (Dinan and Cryan, 2012). High feather pecker hens seem to have different microbiota than low feather pecker hens (Meyer et al., 2013). Rabhi et al. (2020) found, based on cortisol levels, that performers and victims of tail biting were more stressed, compared to neutral pigs. Furthermore, the composition of microbiota differed; more lactobacillus bacteria were present in the microbiota of neutral pigs as compared to performers and victims. Brunberg et al. (2016) discuss two ways to change the microbiota to decrease damaging behaviors: to manipulate the gut microbiota through probiotics and to select pigs with a gut environment that fits favorable microbiota.

Aggressive behavior in young pigs

One of the control questions in Code of good practice for farm animal breeding and reproduction organizations (CODEEFABAR, 2020b) is whether the

pig breeding organization has implemented the element "positive sociability/interaction among animals within the group" in its breeding program. Aggressive behavior is performed especially by pigs that meet for the first time and are similar in body weight. Again, identifying the recipient is much easier than identifying the aggressive pig initiating the fight.

To evaluate the genetic background to aggressive temperament, Velie et al. (2009) used the resident−intruder test on growing pigs. They used a simple scoring based on the cumulative number of attacks over two tests with a score between 0 (the pig never attacked) and 2 (the pig attacked in both tests). The trait was analyzed as categorical using a threshold model. According to this study, attacking an intruder has a low heritability ($h^2 = 0.1$), and the latency to the first attack is not heritable.

To record skin lesions is easier than to study aggressive behavior (Turner et al., 2009). The number of fresh skin lesions can be counted, for example, immediately before and 24 hours after mixing. Number of lesions after mixing and in stable groups are both heritable traits (Desire et al., 2015). Lesions located in the front of the body are associated with a high level of reciprocal fighting. Those located in the rear are the result of the receipt of nonreciprocated aggression (i.e., being bullied whilst being chased or attempting to retire from a conflict). The total number of lesions had a heritability of 0.2 in the study by Turner et al. (2006b). When distinguishing between different parts of the body, the heritability for lesions in the rear was lower both when recorded the day after mixing and 3 weeks after mixing (Turner et al., 2009). Again, this indicates that the genotype of victims is different from the genotype of the attacking pigs. To record skin lesions in the front shortly after mixing seems feasible also in a large scale and it could be used to select against both reciprocal and nonreciprocal aggressive behavior (Desire et al., 2016).

Nucleus herds have been used to study aggressive behavior at mixing. Being involved in fights has a moderate heritability ($h^2 = 0.4-0.5$) and so has the delivery of nonreciprocated aggression ($h^2 = 0.3-0.4$). The genetic correlation between number of lesions and being involved in fights was strongly positive in one study (0.7) but ranged from close to zero (0.1, rear) to 0.7 (front) in another study (Turner et al., 2008, 2009). The genetic correlations between bully delivery and receipt were inconsistent between these studies, maybe due to differences in aggression patterns. Turner et al. (2009) suggest selection against pigs that fight and bully others by recording lesions on different parts of the body as different traits. They conclude, based on low and negative genetic correlations between lesions and growth and backfat depth that selection against lesions would not induce correlated changes in production traits.

In theory, pigs who fight allocate less energy to grow. Torrey et al. (2001) showed that Landrace gilts selected for large loin-eye areas were involved in more aggressive interactions after mixing than gilts with smaller

loin-eye areas. Social rank, recorded during feeding, was highly heritable when estimated on entire males at a test station and the genetic correlation between the position in social rank order and growth rate was high (Jonsson and Jørgensen, 1989). Thus selection for a high growth rate could result in increased aggressiveness, which also has been proposed by Schinkel et al. (2003). Pigs with a high direct breeding value for growth rate are more successful during social contests (Canario et al., 2012). They have higher genetic merit for both initiating and winning fights and bullying than other pigs. Furthermore, these pigs are less frequently bullied and have fewer lesions in the rear.

Individual behavior is adapted to the group around the individual and the performance of the individual is dependent on the group mates. Selection for individual performance maximizes the individual's results but not necessarily that of the group the animal is raised in. Bill Muir became interested in aggressive behavior because of its negative influence on production results. His research has shown alternative methods of selection, improving both production and welfare. Muir developed a model to handle an unfavorable correlation between growth rate and competitive behavior without any need for behavioral observations. The model is called "the group model," "the social model," or "the competitive model". The quantitative relation between individual and group productivity was first presented by Griffing (1967) who extended classic population genetic models to include social effects. Based on Griffing's theory, Muir (2005) presented a social model that was further developed by Bijma et al. (2007) to estimate social genetic parameters. The social model for genetic evaluation of growth rate includes not only the "ordinary" direct genetic effect on an individual's growth rate, but also the social genetic effect on all group members' growth rates (Fig. 9.3).

With this approach, two breeding values are estimated for each animal, one describing the animal's genetic ability to grow and the other describing the animal's genetic ability to influence the growth of other animals in the pen, the so-called social breeding value. The correlation between the direct (D) genetic effect and the social (S) genetic effect (r_{DS}) is negative (unfavorable) if the animals compete for limited resources such as food and space. If that is the case, it cannot be recommended to select the animal with the highest breeding value for the direct effect since fast-growing animals are best at the expense of their group members. Instead, animals with rather high breeding values for both the direct and the social effects should be selected. If the genetic correlation between the direct genetic effect and the social genetic effect is positive (favorable) selection of the animal with the highest breeding value for the direct effect will not harm group members. Even so, using both direct and social breeding values in the selection can lead to a larger genetic progress in growth rate than only using the direct breeding value (Rodenburg et al., 2010).

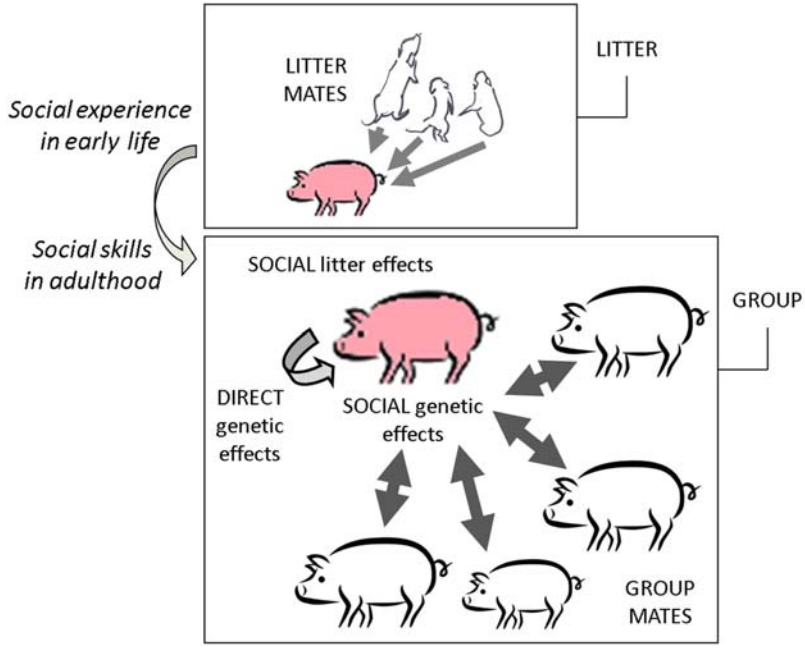


FIGURE 9.3 The direct genetic effect and the social genetic effect on the performance of pigs can be used to estimate direct and social breeding values. The social skills of a pig that influence its social interactions with other group mates are determined by its social experience in early life (achieved in contact with littermates) and its genotype. *Illustration: Laurianne Canario.*

Bergsma et al. (2008) used the social model to study growth rate. The results indicated that pigs cooperate rather than compete ($r_{DS} = 0.2$). In another study, the correlation was estimated at -0.1 (Canario et al., 2010). Chen et al. (2009a) found a range from negative to positive social interactions in different American populations ($r_{DS} = -0.4 - 0.7$). This confirms that the genetic background of aggressive behavior varies between pig populations. Furthermore, Nielsen et al. (2018) found that the correlation between direct and social effects differed between male and female pigs reared in sex-sorted groups.

What characterizes pigs with high breeding values for the social genetic effect? From a Swedish data set, we have both behavioral and lesion score data on the animals and can compare pigs with high and low breeding values for direct and social effects on growth rate (Canario et al., 2012). We simulated different situations in which the pigs displayed negative social interactions (genetic antagonism between own growth and the growth of group mates; $r_{DS} < 0$), neutral social interactions ($r_{DS} \sim 0$), or positive social interactions (genetic mutualism between own growth and the growth of group

mates; $r_{DS} > 0$). Pigs with high social breeding value at mixing, referred to here as social pigs, can be described as follows:

- When the social environment induces genetic antagonism, social pigs are genetically predisposed to lose fights and to be involved in bullying and being bullied more frequently than others.
- When the genetic antagonism is very strong, social pigs initiate and win fewer fights, probably because the dominant pigs defend their privileged access to food.
- When social interactions are neutral, social pigs lose more fights and receive more aggression.
- When the social environment induces genetic mutualism, social pigs initiate more fights. They actively participate in the establishment of dominant relationships, by being more challenging.
- When genetic mutualism is very strong, social pigs initiate and win more fights and bully others more. That means they adopt a more aggressive strategy.

Under both genetic antagonism and neutral interactions, social pigs accumulate fewer lesions in the front and more lesions in the rear. Under genetic mutualism, there is no difference in lesion scores between more or less social pigs. Three weeks later, social pigs do not differ from others regarding lesions in the front but if the social environment induces genetic mutualism, they have more lesions in the rear, which is a sign of involvement in both reciprocal fighting and receipt of bullying. Social pigs spend less time standing, which indicates that they are calmer (Canario et al., 2012).

If pigs are selected for both the direct and social effects on growth rate in a situation of neutral or positive interactions, social pigs will initiate more bullying immediately after mixing. When the dominance order has been set, they will be less willing to fight and at greater risk of being attacked (Canario et al., 2012). Aggressive behavior at mixing is a necessary adaptation to the rearing environment that does not hamper pig results and is probably not correlated to aggressive behavior later, in stable groups. Aggression among pigs is of two broad kinds: intensive fighting during a brief period when unfamiliar pigs are mixed and longer-term competition over feed or other limited resources to maintain hierarchy. Hong et al. (2018) recorded pigs' behavior at three occasions. One day after mixing and 1 month after mixing, pigs with high social breeding values spent more time on feeding and less time on aggressive behavior than pigs with low social breeding values. The results were, however, less clear 2 weeks after mixing. Thus it is important to consider an appropriate time window in pig life if one intends to select directly on a social behavior trait.

Pigs with high and low social breeding values for growth from a one-generation selection experiment were compared by Camerlink (2014) and Reimert (2014). They concluded that pigs with high social breeding values

tend to be less fearful and less affected by stress. Such pigs also seem less predisposed to tail biting (Camerlink et al., 2015). Wu et al., (2019) performed a genome-wide association study of pigs with high and low social breeding values and found more than 50 candidate genes for the social breeding value. One of them, GABRR2, has previously been found to influence stress response and aggressive behavior in animals.

Unfavorable correlations between the direct and the social genetic effect have been found in trees, quails, and several laying hen populations (Bijma, 2011). Bouwman et al. (2010), on the contrary, found no direct-social correlation on early growth in pigs. The group model assumes that an animal's social effect is similar on all other pigs in the group, regardless of whether they are relatives or not. Only cooperation between relatives fits well into Dawkins' story of *The Selfish Gene* (Dawkins, 1976), but pigs rely on familiarity rather than genetic relatedness and they are not able to recognize relatives if they have not been raised together (Bekoff, 1981). Are there "helpful" pigs, and if so, why? Natural selection would result in selfish individuals that are successful at the expense of others, not in successful groups. This is observed, for example, in wild plants that compete for light, but breeding is not equal to natural selection. Maybe "helpful" pigs are merely pigs not disturbing other pigs? Pigs genetically predisposed for eating and staying calm without spending time and energy on bothering group mates would get high breeding values for own growth and for pen mates' growth. That strategy is probably only successful if feed is not a limited resource.

It would be interesting to study how general the breeding values for social effects are in different environments with more or less limited resources. With a group size ranging from 5 to 15 animals per pen, the social genetic effects were diluted in larger groups (Canario et al., 2010). This can be handled by including a dilution factor in the model (Bijma, 2010). Turner et al. (2001) suggest that in very large groups, social strategies depend less on social hierarchy since all pigs do not recognize each other. It can be assumed that the behavioral response to selection for growth will be quite different with different group sizes and also with different feeding systems that trigger more or less competition.

Pigs change in physiology, cognitive abilities, and social behavior over the course of their lifespan. Interactions with the environment shape an individual phenotype, in its coping ability and susceptibility to stress. Part of the explanation is found in recent research highlighting that the genetic background of shown behavior not only depends on the DNA sequence and structure, but also on the regulation of gene expression that is referred to as epigenetics. Epigenetics participates in a nongenetic transmission of behavior. Hence, the environment, genetics, and epigenetics shape the adult phenotype. Although several studies address transgenerational epigenetic inheritance, only a few results provide a basis for the inheritance of acquired phenotypes through epigenetic mechanisms, and making the proof is not easy. Nevertheless, reviews by David

et al. (2019) for livestock, Kasper et al. (2020) for pigs, and Guerrero-Bosagna et al. (2018) for chicken indicate that epigenetic marks present on the DNA sequence can be transmitted through several generations. In particular, the impact of environmental influences is mediated by epigenetics that alters physiology, metabolism, and movement of individuals, and this may influence the social ability of young and adult pigs.

A possible future of animal production may be to use epigenetic effects to influence gene expression. Assume epigenetic effects on genes involved in aggressive behavior can be transmitted from generation to generation. Further, assume that a good treatment of parents early in life influences DNA methylation of their offspring in such a way that alleles associated with aggressive behavior are turned off. Then providing young AI-boar candidates in sire lines with the very best environment (enriched environment, tailor-made feed, etc.) could make a huge difference for the welfare of all pigs raised for slaughter. The number of these young boars is relatively low, and thus it would be affordable to give them an enriched, more expensive environment.

Aggressive behavior in sows

Sows that have been housed in farrowing pens fight during the first 24 hours after weaning, when mixed with other sows. Sows should be loose housed for welfare reasons, but fighting can be detrimental for low-ranked sows. In FAWC's report on the Welfare Implications of Animal Breeding and Breeding Technologies in Commercial Agriculture, it was stated already in 2004 that "FAWC is aware that selection for temperament is becoming increasingly important ... for species such as pigs and laying hens where a move away from close confinement systems, driven by either legislation or market forces, has revealed the importance of behavioral traits such as reduced levels of aggression (FAWC, 2004)". Bunter et al. (2015) used the group model on loose-housed pregnant sows and estimated direct and social effects on litter size. The social heritability for litter size was very low (<0.01), and the correlation between the direct and the social effect was close to zero. The link between social behavior and growth rate seems more direct (assuming competition for feed) than the link between social behavior and litter size, but embryonic loss may be influenced by stress caused by aggressive behavior. It would be interesting to use the group model also for traits like weaning to estrus interval and pregnancy rate.

In a Danish field study of pregnant sows, all aggressive interactions during the first 30 minutes after mixing were recorded (Lövendahl et al., 2005). The identity of sows delivering and receiving attacks was noted. The model included two genetic effects, one describing the capacity to attack and the other describing the predisposal for being attacked. Repeated measures from all dyads (all pairs) of sows in the pen were analyzed. Performing aggressive behavior was heritable ($h^2 = 0.2$), whereas being a victim had a heritability close to zero.

A simpler way of recording (and analyzing) is to just record the sum of attacks given by each sow and base the genetic evaluation on that sum. This gives a higher heritability and would work well in practice. In a nucleus herd of 100 sows, approximately 12 sows are weaned every third week. One hour's work every third week would allow the farmer to mark the sows and record aggressive behavior and then the breeding organization could include aggressive behavior of sows in the genetic evaluation and select against it, to improve welfare. Hellbrügge et al. (2008) recorded aggressive behavior of pregnant sows when mixed in small groups during washing, before entering the farrowing unit. Eighteen percent of the sows were aggressive, and the heritability was estimated with a threshold model at 0.3. They also found favorable genetic correlations between aggression in groups and maternal behavior (less aggressive—stronger reaction to the sound of a screaming piglet).

Several markers and some genes governing aggressiveness have been found in rodents, and with a candidate gene approach based on genes with a known impact on the hypothalamic-pituitary-adrenal (HPA) axis, associations between skin lesions and the gene NR3C coding for a glucocorticoid receptor as well as the gene AVPR1B coding for a vasopressin receptor were found in pigs (Muráni et al., 2010). Terenina et al. (2012) used the same data set and found markers indicating that both dopamine- and serotonin-related genes are involved in the genetic variation of aggressive behavior.

Selection of pigs based on breeding values estimated with best linear unbiased predictions (BLUP) have during the last forty years led to huge progress in many traits. Phenotype records together with known identity and relationships between animals constitute the base for BLUP breeding values. Genomic breeding values (G-BLUP) are estimated based on a combination of genomic data from single-nucleotide polymorphism markers from all pigs and phenotype records from pigs in a reference population. Such breeding values can be estimated with higher accuracies than traditional BLUP values, and therefore less phenotype records are needed for estimating G-BLUP values with high accuracy, as compared to ordinary BLUP values. Genomic selection is especially relevant for traits that are complicated and expensive to record, which is true for the aggressive behavior of sows and many other behavioral traits. In genomic selection, knowledge about markers from genome-wide association studies can improve the accuracy of genetic evaluation of behavioral traits even further. Even if genotyping of enough numbers of pigs for the reference population is not done today, it could become affordable with a decreasing genotyping price.

Maternal behavior

Piglet mortality is a severe problem in pig production related to the ongoing selection for larger litters and leanness. Genetic studies on the behavior of newborn piglets are scarce, but several genetic studies on maternal behavior

have been performed. The long-term goal of most of these studies is to decrease piglet mortality by breeding.

Maternal behavior starts already before farrowing, with nest building. This behavior makes the sow different from other ungulates. Nowak et al. (2000) wrote "The fact that nest building has been retained in spite of domestication indicates how robust this maternal behavior is". Nest building is performed to provide the piglets with shelter and comfort, and particularly to keep them warm. This is especially important for small piglets due to the greater surface-to-body mass ratio resulting in higher heat losses. Nest building is a heritable trait in mice (Lynch, 1980). Ocepek and Andersen (2018) showed that the sow's nest building activity is associated with lower proportions of both starved and crushed piglets.

The duration of farrowing influences piglet survival and the risk of dying during or shortly after farrowing is greater for piglets born late (Canario et al., 2009). Farrowing duration is therefore a potential selection trait, but it is very difficult to record on a large scale. For a proper assessment, the observer should stay beside the sow and at the same time avoid disturbing the farrowing. Cameras and image analysis may facilitate future selection for a short farrowing. There is a genetic variation in farrowing duration (but the heritability is low when recorded in nucleus herds; $h^2 \leq 0.1$) and it is correlated with litter size and number of stillborn (Holm et al., 2004). No genetic relationships between farrowing duration and behavior are reported in the literature.

Sows are sometimes aggressive toward their newborn piglets. Infanticide (also called savaging) is associated with endocrine changes during farrowing, farrowing experience, environment, and genotype. Savaging piglet is a tricky trait to analyze, and the range of heritability estimates is large ($h^2 = 0.02-0.9$; Knap and Merks, 1987; Van der Steen et al., 1988; Gäde et al., 2008). Although rarely included in the genetic evaluation, there is an ongoing selection against this trait at the farm level. Besides, natural selection helps the breeders; often, there are no piglets alive to select in affected litters. Single genes with a large influence on this defect have been found, and many of them seem to interact. Several QTL have been detected on different chromosomes, among which a promising QTL on the X chromosome has been found in a Large White Duroc × Erhualian population (Chen et al., 2009b). Bauer (2019) found associations between savaging and genes involved in the regulation of dopamine, vasopressin, and oxytocin levels. Some QTL regions found in pigs have corresponding regions in humans and rodents that harbor genes controlling anxiety, bipolar disorder, or coping behavior (Quilter et al., 2007), and some mitochondrial genes are differentially expressed when savaging and not savaging sows are compared. This is interesting because the mitochondria contain DNA that is inherited solely through the mother. Several of these genes are related to neural pathways.

Sows' communication with piglets while being active seems to be a predictor of low piglet mortality and high litter weight gain. Ocepek and Andersen (2018) recorded communication as sniffing, grunting, and nudging. The authors concluded that sows that communicate more with piglets are more aware of their presence and may protect them to a larger extent. Crushing by the sow is one of the most common causes of piglet mortality. The introduction of farrowing crates was primarily aimed at reducing crushing. Crates also facilitate good hygiene and protect the farmer from aggressive sows. There is growing contest against this production system that induces higher stress in sows as compared to farrowing pens without crates (Jarvis et al., 1997), and we must use alternative ways to prevent crushing, such as selection for improved maternal behavior.

Half of the crushed piglets are probably healthy piglets, but to identify the cause of death accurately on a large scale, for example, in nucleus herds, is very difficult. The heritability for percentage of crushed piglets was estimated at 0.06 on data from a research herd (Grandinson et al., 2002) and at 0.04 on data from nucleus herds (Grandinson et al., 2003). The sow's reaction to the sound of a screaming piglet has been studied by several researchers. The postural reaction of the sow is heritable (Grandinson et al., 2003). Using a questionnaire to the farmers, Vangen et al. (2005) estimated the heritability of sow carefulness in two populations at 0.1 and 0.2.

The sow's reaction to a screaming piglet is genetically correlated to piglet mortality; stronger reaction—lower mortality ($r_g = -0.2$; Grandinson et al., 2003). But Hellbrügge et al. (2008) found a stronger correlation between piglet mortality and sows' reaction to pop music ($r_g = -0.3$) than to piglet scream. Our interpretation is that a test of this kind reveals the sow's maternal ability, regardless of whether it believes the sound comes from its own piglets or not.

The relationship between a sow and its piglets seems to rely upon piglets' bond to their teats rather than strong sow−piglet bonds. Even so, there is a variation in sows' reactions to being separated from their piglets. Hellbrügge et al. (2008) estimated the heritability for this trait at 0.1 both at farrowing and 3 weeks later. The reaction was stronger at 3 weeks and the genetic correlation to piglet mortality was rather high ($r_g = -0.4$). Gäde et al. (2008) evaluated maternal ability 3−5 days after farrowing on a scale from 1 to 5 by combining several aspects: behavior during farrowing, behavior during lying down (carefulness), nursing behavior (position during nursing), reaction to screaming piglets, and number of crushed piglets. They estimated the heritability for this combined trait at 0.05. Canario et al. (2007) have observed breed differences in nursing behavior between Large White and Meishan sows, and Vangen et al. (2005) found some genetic variance in nervousness during suckling and willingness to expose the udder in a field study. Stratz et al. (2016) defined good nursing behavior as not lying on the udder, feeding piglets until satiation, and having a well-developed udder with functional

teats. This nursing trait, assessed by farmers, had a heritability at 0.10 and the genetic correlation with piglet weight at weaning was very high.

Environmental effects mediated by maternal care exist (Francis et al., 1999), and the behavior of female piglets can be influenced by the sow's behavior. Besides genetic transmission, female offspring can learn from the maternal behavior of their nursing dam and use such early social learning later when they raise their own progeny. Cross-fostering showed that the progeny of mice exhibiting low maternal behavior, but nursed by mice exhibiting high maternal behavior, display high licking patterns of their own progeny (Champagne and Meaney, 2007). Cross-fostering designs also showed direct effects of maternal care on the behavioral and neuroendocrine responses to stress in the progeny, which supports the existence of an epigenetic underpinning mechanism (Francis et al., 1999). Deficient maternal behavior had a negative effect on the cognitive behavior of the offspring. This effect was, however, reversed by exposure to an enriched environment during the peripubertal period (Bredy et al., 2004; Champagne, 2008).

Differences found in the hippocampus of the offspring from rat dams exhibiting high and low levels of maternal care could be reversed by cross-fostering (Weaver et al., 2004). Such increased hypothalamic methylation increases HPA responsivity in the offspring of dams enduring gestational stress (Mueller and Bale, 2008), making them less sensitive to environmental sources of stress. It would be interesting to study epigenetic effects in sows with high versus low maternal care and their offspring, to evaluate to what extent the conclusions obtained in rodents hold also for pigs. If DNA methylation plays part in the expression of maternal behavior in sows, studies should be performed not only on the alleles in the genome, but also the methylation ought to be studied.

Given the strong impact of maternal behavior on the different inherited factors, fostering the potential future reproducers (given their genetic potential) to dams with good maternal abilities would certainly promote the transmission of "good" epigenome and behavioral skills to the next generations. Also, providing sows with an enriched environment may influence DNA methylation in a positive manner for their own maternal skills and the development of their offspring. Moreover, appropriate handling of the offspring should be implemented, given that separation from the dam may have either positive or negative impact on the offspring development, depending on the magnitude of the stress imposed and the time window when it is applied, and the epigenome acquired from their dam, which may also change over the lifespan.

Many sows are kept in crates during farrowing and lactation, but in some European countries farrowing crates are forbidden due to welfare reasons. Furthermore, sows in outdoor production are never in crates. The ongoing structural trend toward less but larger (and often multinational) breeding companies raise the question whether there are important $G \times E$ for maternal

behavior in systems with crates, farrowing pens without crates, or huts outdoors. Baxter et al. (2011) compared two indoor sow lines, one selected for high survival from birth to weaning, and a control line selected for average piglet survival. All studied animals were born and reared on outdoor commercial units. They were later compared in two environments, indoors in pens without crates and outdoors. In both environments, the control sows crushed more piglets than the high-survival sows. Thus selection for improved piglet survival limits the crushing behavior regardless of the environment. Indoors, the high-survival sows did, however, show more aggression toward the piglets and savaged more of them as compared to the control sows. No such difference was observed outdoors. Probably, the high-survival sows reacted more strongly to the new indoor environment than the control sows, and this reaction was expressed as aggressiveness toward the piglets. Thus when selecting animals for improved maternal behavior, the production system should be considered.

Piglet survival and growth depend both on the genotype of the piglets and the genotype of the sow. Leenhouwers et al. (2001) compared newborn piglets with high and low direct breeding values for survival. The difference in breeding values was not reflected in early postnatal behavior. However, sows with high maternal breeding values for piglet survival gave birth to piglets that took a shorter time to reach the udder and suckle for the first time, as compared to sows with low maternal breeding values (Knol et al., 2002). It could be assumed that this difference reflects differences in sow behavior and calmness during farrowing. Engelsma et al. (2011) estimated correlations between the breeding value for maternal ability (based on a percentage of liveborn piglets that survived) and various behavioral traits. Around farrowing, sows with high breeding values tended to perform less postural changes and be less active, but these correlations were not significant. During lactation (week 1−2), sows with high breeding values spent more time lying laterally and less time sitting, thus facilitating piglets' access to the udder. They also performed less postural changes that may limit crushing.

Questionnaires in which the farmers summarize their observations of sows' behavior during farrowing and lactation can be an alternative to time-consuming behavior tests or video recordings under field conditions. Vangen et al. (2005) used a questionnaire where farmers were asked to assess maternal behavior of Norwegian and Finnish Landrace sows on a scale from 1 to 7. Most of the results were very consistent across countries. The highest heritabilities were found for the sow's reaction to piglets screaming when handled ($h^2 = 0.1-0.2$) and how often the sow showed fear during routine management ($h^2 = 0.1-0.2$).

There is variation in maternal behavior between breeds. At farrowing, Meishan sows spend more time standing, manipulating straw, and exploring the floor, and they have more nose contact with the piglets than Large White sows (Canario et al., 2007). Meishan piglets show higher vitality at birth

than Large White piglets. When crossbred F1-piglets of these breeds are compared (i.e., similar piglet genotype for different maternal genotypes), piglets born from Meishan sows spend more time sleeping in contact with the sow's udder but are less eager to suckle than piglets born from Large White sows (Fig. 9.4). This emphasizes the interaction between maternal and offspring behavior.

A study design with both purebred and crossbred piglets in the same litter further highlights the mechanisms of interactions between generations. For this purpose, purebred animals were inseminated with a mixture of semen from both breeds (Dauberlieu et al., 2011). At birth, both purebred and crossbred piglets born from Meishan sows take less time to reach the udder than piglets born from Large White sows, for which purebred Large White piglets

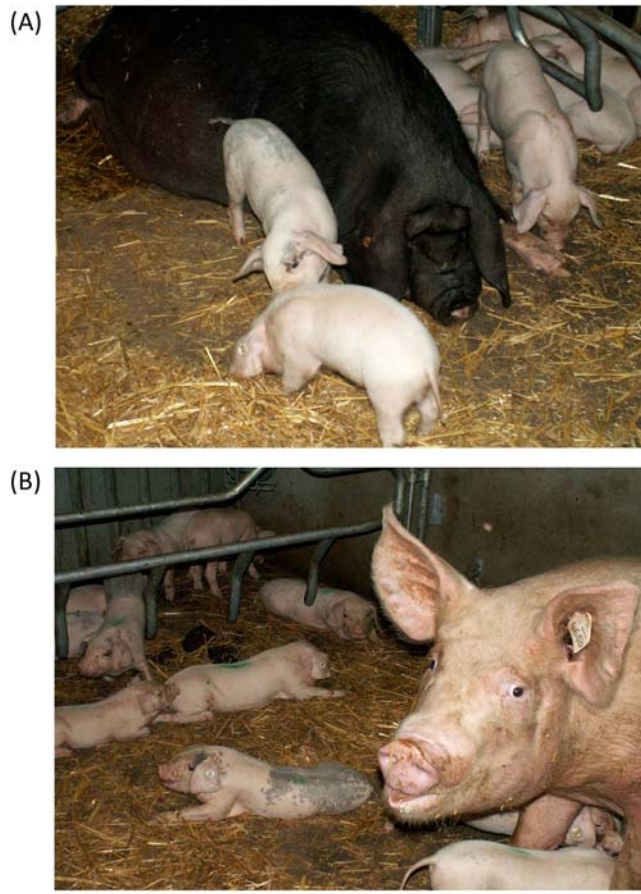


FIGURE 9.4 Comparison of maternal behavior between Meishan (A) and Large White (B) sows with crossbred F1-litters. *Photograph: Laurianne Canario.*

are the slowest. The purebred Large White piglets are quicker to reach the udder when raised by Large White dams that lie laterally for longer times at farrowing. Purebred and crossbred piglets born from Large White sows differ more than those born from Meishan sows in behavior and growth over the first days after birth.

Sows selected for high lean-tissue growth rate are less active at farrowing and more responsive to piglets than sows selected for low lean-tissue growth rate (McPhee et al., 2001). At first sight, this may be interpreted as good for piglet welfare because of a decreased risk of crushing. However, the low activity reveals a poor welfare of the sow with negative consequences for the piglets. The probability of stillbirth is higher in piglets born from sows that are lying for much of the time, because the low activity is associated with more farrowing difficulties (Canario, 2006).

Only weak genetic trends could be found in maternal behavior in French Large White sows that have been selected for prolificacy and lean growth rate for decades. By using frozen semen, two types of sows could be compared. Crosses between old-type or modern-type sires and modern-type sows were used to create two populations that were studied in the same environment and at the same time for several generations (Canario, 2006). The lines did not differ regarding the cause of piglet neonatal mortality (crushing by sow and piglet starvation) and infanticide was rare in both lines. The reaction of the sow when separated from the litter did not differ between the two lines. The sow's response to nose contact initiated by piglets was stronger for modern-type sows than for old-type sows. The largest difference was observed for the postural activity of the sow at farrowing. Modern-type sows were lying for a longer period after the onset of farrowing, which was associated with greater farrowing difficulties (Canario, 2006). In line with this difference, both stillbirth and piglet weight were higher in modern-type litters (Canario et al., 2007).

A complicating factor when selecting for improved maternal capacity is that cross-fostering is widely practiced, even in nucleus herds. The breeding value for maternal capacity can be calculated as the maternal genetic effect on piglet survival related to the sow that fosters the piglets, regardless of whether it is the biological mother or not. It is, however, not obvious whether piglets dying shortly after being moved should be regarded as a failure of the biological or the foster mother. An advanced model can include three genetic effects: the maternal effects of the biological and the foster mother and the direct effect of the piglet. Such a model puts very high demands on data structure and computer capacity and is difficult to use in routine genetic evaluation.

Freedom from fear and distress

High levels of fear and anxiety are disadvantageous for pigs as well as farmers. Fearful and aggressive animals are more difficult to handle, and they can

be a risk for the handler. Fortunately, pigs are not very aggressive toward humans. All caretakers know they should never turn their back to a boar, but we are not aware of any genetic studies on human-directed aggressive behavior of boars. We have, however, noted that the word "aggressive" is used in the marketing of sire lines. A sire line can, for example, be described as "aggressive AI boar excellent for semen collection," which could reflect behavioral differences between lines.

Fearfulness

Domestic pigs meet humans every day, and there is usually no escape. Fearfulness thus could have a large effect on pig welfare, but in general pigs seek contact with the caretaker rather than avoid him/her, unless the caretaker behaves "badly". There is an ongoing threshold selection against fear at the herd level that is partly a natural selection and partly an active selection performed by the breeder. This selection has always been an important part of the domestication process. In case of unfavorable genetic correlations between fear and production traits, natural selection may however not be enough to hinder a negative development. There are indications that selection for lean growth rate results in a more excitable temperament in pigs (Grandin, 1992). Leaner pigs are more stressed at transportation, balk more, and are more difficult to drive through races at the slaughter plant than fatter pigs (Grandin, 1998). Scott et al. (2000) compared a lean and a fat Landrace line. The lean animals were more reluctant to voluntarily approach a human.

The heritability for fear of humans has been estimated based on growing pigs, gilts, and sows. The response of pigs to humans tends to be more heritable when the trait is recorded on animals isolated from the group. The heritability of voluntary approach to a human (time to first contact) was estimated at 0.4 based on isolated gilts (Hemsworth et al., 1990) but the heritability was only 0.1 when based on gilts in groups (Hellbrügge et al., 2007) and 0.0 when based on growing pigs of both sexes in groups (Velie et al., 2009). The comparison must be done with caution as the animals belong to different populations and also differ in age.

Easy to handle

In growing–finishing pigs, the assessment of "easy to handle" can be performed in association with a routine procedure that takes more time if the pigs are not cooperative. Variation between breeds exists in this trait. Terlouw et al. (1997) showed that Large White pigs react more strongly to human presence than Duroc pigs. Lepron (2006) compared three genotypes: a Large White line, a line intensively selected for loin and ham muscle development (Meat line), and a line with Meishan and Large White genes. The Meat line pigs took longer time to exit the pen, run more frequently at

weighing, and required more human interventions when moved out of the pen, as compared to the other lines.

Moving pigs is a common task on any pig farm. Pigs are moved between pens, to the scale at weighing, to the boar at mating, and on/off the trailer when transported. This work is time-consuming, and there might be an optimal tameness of pigs. Very flighty animals run off in the wrong direction, jump over barriers, and can create chaos. Very tame animals move slowly because they want to come close and interact with the caretaker instead of walking in front of him/her. Day et al. (2002) found that pigs exposed to a pleasant-handling treatment took more time to move out of a pen because of reduced fear of humans. Grandin (1989) also reported that handling becomes difficult when pigs are overly tame. The Meat line pigs in Lepron's study (2006) exhibited behaviors indicative of fear, stress, and excitation, such as bouts and U-turns. Thus they seemed more nervous than the other two lines. Large White pigs also showed some distinctive reactions to handling. They vocalized more and their heart rate increased more than that of the Meat line pigs. Pigs from the line with Meishan genes appeared to be the least stressed when moved out of their pen.

Response to weighing is a heritable trait in young pigs: the heritability for score for restlessness in the scale was estimated at 0.2 (Holl et al., 2010) and for time to exit the scale at 0.1−0.2 (Jones et al., 2009). The genetic correlation between restlessness and growth rate was negative ($r_g = -0.4$, more restless − lower growth rate) and the corresponding correlation with leanness was positive ($r_g = 0.1 - 0.2$, more restless—leaner). The genetic correlation between exit time and growth rate was positive ($r_g = 0.1$ n.s., longer time—higher growth rate) and the corresponding correlation with leanness was negative ($r_g = -0.2$, shorter time—leaner). Selection for growth rate would thus lead to less excited animals whereas selection for leanness would lead to more excited animals. D'Eath et al. (2009) recorded handling scores during weighing. Aggressive behavior at mixing at 10 weeks was genetically associated with handling scores at 22 weeks; pigs that fought and delivered nonreciprocal aggression were more active during weighing, whereas pigs that were bullied needed more encouragement to enter the scale. When sow behavior was recorded with a questionnaire in two populations, protests by the sow when moved to the farrowing pen was heritable in one of them ($h^2 = 0.2$) but not in the other (Vangen et al., 2005).

Lactating sows' aggression toward humans

Aggressive behavior of sows is more often observed when lactating sows are kept outdoors or group housed in family pens than in ordinary pens indoors (Wallenbeck et al., 2009). Aggressiveness may be less of a problem for the caretaker if sows are kept in stalls or crates. Even so, aggressive behavior of sows is not regarded as a big problem in Sweden, where stalls for dry sows

and farrowing crates have been forbidden for many years. Less than 10% of the sows reacted aggressively in a piglet-handling test where the breeder entered the farrowing pen, lifted a 4-day-old piglet, and squeezed it gently until it screamed (Grandinson et al., 2003). Avoidance of humans was also recorded in the piglet-handling test and 70% of the sows neither moved forward nor withdrew from the breeder. The heritabilities of aggression toward humans and avoidance were estimated at 0.1 in that field study. Piglet mortality was not genetically correlated to aggression but to avoidance of humans ($r_g = 0.4$, more avoidance—higher mortality). It must be noted that the genetic analyses included only 1100 observations and the standard deviations of these correlation estimates were very large.

When aggression of lactating sows toward humans was recorded with a questionnaire to farmers, the heritability was estimated at 0.1 (Vangen et al., 2005). The heritability of fear of humans was estimated at 0.1−0.2 in the same study. We do not know if these two traits are genetically correlated but admit that the thought of meeting a large sow who has no fear of humans can be somewhat alarming, especially in outdoor production. In the study by Baxter et al. (2011), sows selected for higher piglet survival were both more aggressive toward their piglets and toward the humans handling their piglets. They were also more reactive animals, which suggests that farrowing was a period of acute stress for these sows.

Pigs' ability to handle stress

Many studies of stress in pigs have been performed, but the motives have often been more related to meat quality than to pig welfare. The genetic selection for high production has probably contributed to a reduction of HPA axis activity and consequently to a decreased capacity of modern, high-producing animals to maintain their production despite environmental changes (Mormède and Terenina, 2012). The acute response of the HPA axis can be studied by monitoring the release of ACTH, and cortisol levels are often used to measure chronic stress when assessing animal welfare (Wiepkema and Koolhaas, 1993). The stress response, measured by the response to ACTH, is heritable. Gene expression studies have indicated candidate genes for differences in ACTH response (Hazard et al., 2008; Jouffe et al., 2009). But the association between ACTH response and behavior is seldom investigated. Désautés et al. (1997) showed that Large White pigs had higher poststress ACTH levels than Meishan pigs after an open-field test. They also showed significant correlations between vocalization and locomotion scores and poststress ACTH levels, suggesting that these measures reflect the level of reactivity to the environmental challenge and that they may have a common genetic background.

Engelsma et al. (2011) used the open-field test and found that sows with higher breeding values for piglet survival were more explorative; they

manipulated the floor and the walls for a longer time than sows with low breeding values. In a small study where Meishan (MS) and Large White (LW) gilts were compared, a test series including the reaction in an open-field test (5 minutes of exploration), the reaction to a novel object (a bucket descended from the ceiling and left on the floor for 5 minutes), and finally the reaction to an unfamiliar human entering the open field and standing there motionless for 5 minutes was used (Canario et al., 2009). In the first challenge, the MS females that stand motionless were the ones with the highest litter growth later on. The LW females that explore less were the ones with the lowest stillbirth rate and piglet mortality later on. In the last challenge, the MS gilts that remained standing all the time had higher piglet growth and the LW gilts that showed higher latency to first move had higher piglet survival. Thus gilts' ability to handle stressful situations determines their maternal ability later in life but these relations differ between breeds.

Low ability to handle stress, so-called porcine stress syndrome is caused by a mutation in the locus for the ryanodyne receptor gene RYR(1). The gene is often called the halothane gene. The mutated allele, n, is recessive. It is associated with high leanness and high susceptibility to the development of stress. Stressful stimuli trigger a potentially lethal condition known as malignant hyperthermia in homozygous nn pigs and the n allele has been eradicated from several populations. As all crossbred pigs raised for slaughter are heterozygote, the problems of stress sensitivity and mortality are mainly seen in the purebred sire line. Even so, according to Murray and Johnson (1998), Nn pigs also die more often during transport than NN pigs. Furthermore, Nn pigs were less active than NN pigs in an open-field test (Fàbrega et al., 2004). Under commercial conditions, no behavioral differences were found between NN and Nn pigs of various ages in traits like frequency of resting, nonsuckling activity, suckling/eating activity, and social interactions (Fàbrega et al., 2005). The example of the halothane gene is interesting because it was one of the first major genes that could be used in breeding programs. Some breeding organizations decided to refrain from the n allele and its high leanness due to welfare reasons as long ago as the 1980s, while other breeding organizations continued to use the n allele without ethical concerns. One of the control questions in Code of good practice for farm animal breeding and reproduction organizations (CODEEFABAR, 2020b) is whether the pig breeding organization has implemented the element "Elimination of stress susceptibility" in its breeding program.

Terlouw et al. (1997) and her colleagues have compared different breeds with regard to behaviors that reflect the ability to feel or cope with stress as well as stress hormone levels and meat quality. Duroc pigs touched humans more often than Large White pigs did in a human-exposure test. However, when doing so, the Duroc pigs had higher heart rates than the Large Whites. Touching the man or not in the test depends on the fear the man induces in the pig and the pig's motivation to have contact with the man. It is not

known whether the Durocs were less fearful or more motivated to touch the man as compared to Large Whites. Anyway, the response in this test (heart rate) had less effect on meat quality in Durocs than in Large Whites. Terlouw (2005) concludes that the impact of stress response on meat quality is breed dependent. Maybe the same is true for the impact of stress response on welfare?

Freedom to express normal behavior

In the fifth freedom of FAWC (1992), it says that animals should have the freedom to express "normal behavior". In line with that, demands for the ability to express normal or natural behavior are found in animal welfare laws, and in organic farming, natural behavior is a key issue (Lund et al., 2004). The Swedish Animal Welfare Act (1988), for example, states that "Animals shall be accommodated and handled ... in such way as to ... permit natural behavior". The international standards for organic animal husbandry state that "systems that change from conventional to organic production require a conversion period to develop natural behavior" and that "organic livestock husbandry is based on ... respect for physiological and behavioral needs of livestock" (IFOAM, 2005). The definition of the natural behavior of domestic animals is, however, problematic. Are all behaviors performed by wild boars "natural" for domesticated pigs? Segerdahl (2007) proposes that the natural behavior of domesticated animals is a philosophical rather than a biological concept. During domestication, animals adapt to agricultural environments via interaction with caretakers, and this genetic change will influence their behavior.

The normal or natural behavior concept is further complicated by the fact that such behaviors can be "bad behaviors," leading to goal conflicts when aiming for improved welfare. Aggressive behavior has a genetic background, as described earlier. To attack a foreign pig could be regarded as a normal behavior in confined situations where contact cannot be avoided. The consequence of the freedom to express such behavior has only been studied from one perspective, that is, the perspective of the attacked pig whose welfare is decreased. Whether having the freedom to express an aggressive behavior is related to higher welfare has not been studied. Assessment of positive emotions in animals is a complex but growing research field (Boissy and Lee, 2014). High-duration tail movement seems to be an indicator of positive emotions and high-frequency ear movement an indicator of decreased positive emotions in pigs in a study by Marcet Rius et al. (2018). In that study, tail and ear movements were recorded from videos by an observer, but image analysis of videos is feasible (D'Eath et al., 2018) and in the future even phenotyping by sensors may become possible.

One example of a normal or natural behavior is mounting. Mounting is more frequent in entire males than castrates and females, but the entire males

seem to mount other pigs regardless of sex (Rydhmer et al., 2006). According to Hemsworth and Tilbrook (2007), there are breed differences in male sexual behavior and the heterosis observed for libido in crossbred males also indicates a genetic background. In boars used for mating or for semen collection, sexual behavior is, of course, a wanted behavior. In entire males raised for slaughter, on the contrary, mounting is an unwanted behavior. Excessive mounting leads to injuries (Rydhmer et al., 2006). It would probably be possible to decrease the frequency of mounting by selection, but selection against sexual behavior can hardly be included in any breeding program claiming to be sustainable in a long-term perspective.

The expression of normal behaviors is limited for sows that are highly confined during the lactation period. This is the current situation for sows in most conventional systems in Europe; for the large majority of piglet production, the sow is isolated in a crate. Crates were designed decades ago to limit sows' activity and to slow down their postural changes to limit the risk of crushing piglets. Such a high level of confinement limits the expression of the maternal potential of sows (Pedersen et al., 2013). Routine use of crates is forbidden in some European countries and following the European Citizen's Initiative "End the Cage Age" (see www.endthecageage.eu), loose housing of the sow and its litter in a farrowing pen should in a few years' time become a dominant system. In this context, the use of sows with good maternal ability, capable of rearing many piglets until weaning without crushing them, becomes essential. This can be achieved through the genetic improvement of sows. Comparing the activity level of sows between housings may help assessing the consequences of the environment on sows' health and welfare, and help identifying sows with favorable movement patterns. Second, comparing the associations between sow activity and piglet mortality in different types of pens will provide the necessary knowledge to accompany the transition to a major reduction in the confinement of lactating sows. Accelerometers have been used on sows (Ringgenberg et al., 2010), but in the future, more sophisticated sensors may facilitate these kinds of behavior measurements (Lao et al., 2016; Dore et al., 2020).

New phenotypes with new technologies

It is complicated to verify that current breeding activities are performed with respect of the five freedoms, due to phenotyping limitations. Firstly, many behavioral traits important for welfare are time-consuming to record, for example, aggressive behavior and tail biting. The ongoing digitalization on farms and the fast development of sensors give new possibilities. Image-based recording methods and sensors keeping track of individual animals in a group can become useful in selection. Rodenburg et al. (2019) and Ellen et al. (2019) describe the development of sensor technologies for phenotyping damaging behaviors as a possibility for further improvements of breeding

programs. It could also become possible to record pain by image analysis of facial expression. In a pilot study by Di Giminiani et al. (2016), observers assessed pain based on photos of facial expressions of piglets.

Secondly, pig welfare depends on emotions and mood (e.g., anxiety, willingness to play, depressed and relaxed state), which collectively are termed "affective state". The affective state determines the perception of stress and (by interacting with the immune system) the health and performance of pigs. A well-known example is a curled-up tail associated with "happy pigs". Growing pigs in a barren environment show fewer curled-up tails than pigs in enriched environments (Krugmann et al., 2020). Methods to measure affective state in pigs exist (e.g., Reimert et al., 2013; Lawrence et al., 2018), but methods to assess affective states in the long term are scarce (Baciadonna et al., 2018). Play behavior triggers positive emotions, and tail movement duration increases in a play situation and ear movement frequency decreases, compared to a control situation without play (Marcet Rius et al., 2018). It seems possible to study tail and ear movements of individual pigs on a large scale with cameras and image analysis.

Until now, methods to record affective states have shown pitfalls such as high labor intensity, low repeatability, and low validity, which so far makes them poorly applicable for recording selection traits. Automatic recording of repeated measures in combination with artificial intelligence could, once the methods and models are developed, facilitate recording and increase the accuracy of breeding values based on the new phenotypes. We must, however, remember that these technologies create huge amounts of data and demand bioinformatics skills, powerful computers, and effective data storage.

Pig behavior and sustainable production

Pig production systems should be further developed to become more sustainable, regarding environmental, social, and economic sustainability aspects and goal conflicts between these aspects (Zira et al., 2021). The Code of good practice for farm animal breeding and reproduction organizations has been used by breeding organizations since 2006 and the focus in the early versions of the code was on improving animal welfare. In the latest update (2020), the focus is on achieving the UN's sustainable development goals (SDGs). Sustainable breeding is defined as follows:

> *The extent to which farm animal breeding, as managed by professional organisations, contribute to the production of sufficient, safe, nutritious and healthy food whilst taking care of genetic diversity, resource efficiency, environment, animal health and animal welfare to create "a better world" for future generations. This includes achieving the balance between on the one side food security and on the other side six key subjects: food safety and public health,*

product quality, genetic diversity, resource efficiency, environment, animal health and welfare. (CODEEFABAR, 2020a)

Farm animals are surprisingly absent in UNs descriptions of the SDGs, given their positive and negative environmental, social and economic importance, but according to the code, pig breeding is relevant for several of the SDGs. Next, we will discuss how the pig industry's commitment to fulfill the SDGs can be related to pig behavior and welfare.

Zero hunger (SDG 2). According to the Code, pig breeding reduces hunger by selection for cost-efficient animals, which "makes pork an affordable source of protein for a wider group of consumers". If cost efficient refers to animals with a higher lean growth rate, the unfavorable genetic correlations described in this chapter between growth rate and leanness on one hand and aggressive behavior and tail biting on the other hand could have a negative effect on welfare. A study of Swedish farmers' preferences for breeding goal traits showed that farmers with conventional production wanted to put higher weight on feed conversion and growth of slaughter pigs, as compared to farmers with organic production (Wallenbeck et al., 2016). Nevertheless, more than 70% of farmers from both production systems ranked feed conversion and slaughter pig growth as being of moderate or high importance.

Good health and well-being (SDG3). The Code states that the fulfillment of this goal will be facilitated by "selecting lean and robust animals producing high-quality food with low fat content". Keeping the decrease in ability to handle stress as a consequence of selection for leanness in mind, breeding organizations should monitor potential negative effects in traits important for welfare whenever leanness is among the selection traits. Thus "lean and robust" seems to be a critical combination. An indirect association between SDG3 and behavior is that entire males have leaner carcasses than castrates (and also lower greenhouse gas emissions due to better feed efficiency). With an increased proportion of pigs reared as entire males, selection for decreased aggressive behavior will be more important. A more direct link between well-being of humans and pigs' behavior is a safe and enjoyable work. Sows crushing piglets, tail biting outbreaks, and pig fights leading to injuries on young pigs or sows are examples of unwanted behaviors influencing the work satisfaction of animal caretakers. Another example is sows showing aggressive behavior to caretakers, especially in outdoor production systems. Being attacked by a sow is not only scary, but it can also be dangerous.

Responsible consumption and production (SDG 12). The Code states that "improving the efficiency of pork production by selecting more feed efficient animals producing more high-quality protein and improving animal health, which reduces losses, all of which results in reduced GHG emissions". According to the code, this will lead to a more responsible consumption of pork (and at the same time mitigate climate change). Again, we must keep

the risk of unfavorable genetic correlations in mind. As described in this chapter, selection for low residual feed intake (RFI; more efficient pigs have lower RFI) results in lower physical activity. Welfare consequences of a low activity level are, however, not known.

Concluding remarks

As this review has shown, many behavioral traits important for welfare are heritable and could thus be modified by selection. Furthermore, the ongoing selection for high production seems to influence pigs' behavior. For example, selection for leaner pigs is risky as it increases reactivity to stressors and should not be done without monitoring pig behavior (as well as health and reproduction). The responses to both acute and chronic stressors need to be considered when analyzing the capacity of an animal to adapt behaviorally to the environment, because they refer to different mechanisms of adaptation.

The lowest welfare in pig production is probably found among growing–finishing pigs kept indoors. These animals live in an environment that is extremely boring, especially for such an explorative animal as the pig. Are pigs too smart for their own good? George Orwell knew about pigs' mental capacity when he wrote *Animal Farm*: "The work of teaching and organizing fell naturally upon the pigs, who were generally recognized as being the cleverest of animals" (Orwell, 1945). Could and should we select for calmer, more passive pigs; pigs that are less explorative and therefore less bored pigs? We have not found any heritability estimate for explorative behavior of pigs, but let us assume it would be possible to select pigs for less internally motivated curiosity, and thus less need for a rich environment. Would such a selection be a good way to improve welfare? We would not recommend such a selection. Instead, management systems should be developed, and housing of growing pigs should be improved.

We have described some genetic studies on how easy the pigs are to handle. A question we need to ask ourselves is as follows: Do we select for "easy-care animals" so that we do not have to care? There is a risk that we select animals that can stand a bad environment instead of improving the environment, which is thoroughly discussed by Sandøe et al. (1999) in the article "*Staying Good while Playing God*.". There are no easy answers to these ethical questions. We must work with them again and again, together with all stakeholders, as long as we perform animal breeding.

To increase welfare and at the same time breed for efficient animals, we need to improve breeding programs further. This development should be done together with ethologists, to develop standardized, relevant, and accurate measurements of behavior or other traits related to welfare. Molecular surveys on behavior will greatly improve our understanding of the genes and biological pathways behind behavioral expressions. Genomic selection

(where many markers along the whole genome are used for the genetic evaluation) gives possibilities to select for traits that are difficult and thus expensive to record, like many behavioral traits are. It is, however, important to remember that high-quality recording of phenotypic data is the base for all breeding work, regardless of the method used for genetic evaluation.

Behavioral traits are still rare in pig breeding programs. The only examples we know of is one breeding organization selecting against aggressive behavior of sows and another using both direct and social breeding values for growth rate. In general, we recommend that breeding organizations should define a broader breeding goal, aiming for a sustainable pig production, and they should reflect on the possibility of including pig behavior in the genetic evaluation. Direct selection for behavioral traits is, of course, not the only solution to improve welfare. Often it can be more rewarding to select for improved functional traits. Regarding behavior, we conclude as follows:

- Two research questions that deserve further studies are the role of the microbiome for pigs' behavior, especially tail biting, and epigenetic effects on pig behavior, especially aggressive and maternal behaviors.
- Group housing of sows has now become the standard housing system in many countries. Selection for less aggressive behavior in sows, when kept in groups, is feasible and it could be worthwhile, for welfare reasons.
- Selection against the fear of humans could be relevant, but it is probably more important to choose the caretakers carefully.
- In most countries, sows are still kept in crates during lactation, and this is a welfare issue. Development of farrowing pens that minimize the risk of crushing together with the development of breeding goals aiming for decreased piglet mortality is necessary. Selection for piglet survival, including both direct and maternal effects, should decrease piglet mortality but it is still too high in many populations. Further studies on genetic and environmental effects on the behavior of sows and piglets are important to better understand the biological background of piglet survival.
- It may still be too early to use the group model in the genetic evaluation of growth rate. The optimal weighting of direct and social effects is unclear, and we need more knowledge about pigs' behavior in groups and consequences of selection for social effects before using the group model on a large scale. In the meantime, the consequences of a direct selection against aggressiveness in young pigs ought to be further investigated.

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Chapter 10

Behavior genetics of the horse (*Equus caballus*)

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ABSTRACT
Since the late 1990s, there has been a substantial increase in horse behavior genetics research. This chapter reviews recent work in molecular genetics, pre- and postnatal effects on behavior, the relationship between hair whorls and temperament, and lateralization in the nervous system. These factors are critical to understanding individual differences. Advancements in molecular genetics have identified genes associated with novelty seeking and gaited horse traits. Foal "imprint" training procedures are reviewed, and a gentle method for training foals is presented. Finding appropriate methods for reducing fearfulness in horses has important practical implications. High hair whorls are associated with reactivity, but differences are less apparent in calm breeds. Behavioral asymmetry is a fundamental feature of animal brains. Left-eye systems control avoidance behavior and right-eye systems control approach behaviors. An understanding of the cognitive and perceptual abilities is necessary to ensure horses receive proper training, handling, management, and care.

Introduction

Why do horses behave differently from each other? People have debated this question for centuries. Some believe that horses behave as they do according to inborn tendencies; others think it is because they are taught. A fast-growing body of evidence makes it clear that both sides are partly right. Nature (genetics) endows horses with inborn emotions and traits, and nurture (experience and upbringing) takes these genetic tendencies and molds them as a horse learns and matures (Rankin and Wickens, 2020). Now the debate is centered on how much of horse behavior is shaped by genes and how much by the environment. Although genes play a role in shaping behavior, they never determine it. This is because genes only directly code for proteins, not behavior. However, a change in a single protein code can cause a

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host of downstream effects and may even bring about a distinct phenotype (Keane et al., 2011).

Horses are not very convenient laboratory animals. However, many genetic principles discovered by researchers studying mice or humans in the laboratory are also relevant in horses. For behavioral scientists to tease apart the genetic and environmental influences on complex traits such as temperament, it is important to use large numbers of horses controlled for genetics (age, sex, sire) and environment (stable or pasture housing, type of feed, type of work, caretakers, etc.). Since the second edition, many new studies have been published that show that there are inherited emotional traits (personality) (Librado et al., 2021; Rankins and Wickens, 2020). One trait that has a strong genetic basis is separation stress. When young foals or yearling horses are separated from their mothers, there are stable individual differences in vocalizations and activity (Manrique et al., 2019, 2021). Fearfulness is another emotional trait that has significant individual differences (Christensen et al., 2020).

Molecular behavior genetics

Three studies brilliantly meet these requirements and show the advancements made in the study of behavior genetics. Momozawa et al. (2005) conducted a molecular genetics study on temperament in 136 two-year-old thoroughbred horses (73 males and 63 females) kept at a single training farm in Japan. The horses were at the same stage of training, shared the same training area, and had the same caretakers. Blood samples were collected for DNA analysis, and a questionnaire survey was used to rate the temperament traits of each horse. Interestingly, an association was found between polymorphisms (genes that come in two or more forms) in the dopamine D4 (DRD4) receptor gene and two temperament traits in the horses: curiosity (novelty seeking) and vigilance. The survey had the following descriptions of curiosity and vigilance: "The horse tends to be interested in novel objects and approach them," and "The horse tends to be vigilant about surroundings." Horses with one form of the gene had higher curiosity (novelty seeking). Horses with the other form displayed lower vigilance. The DRD4 gene is also associated with novelty seeking in humans (Benjamin et al., 1996), in primates (Livik et al., 1995), and in dogs (Ito et al., 2004). Momozawa et al. (2005) speculate that diversity in the sequence of the DRD4 gene might influence differences in novelty seeking in various species.

In more recent molecular genetics research, Anderson et al. (2012) discovered mutations in the DMRT3 genes associated with locomotion in gaited horses. A high frequency of DMRT3 mutations was found in horse breeds such as Tennessee walking horse, Missouri fox trotters, Standardbred trotter, and French trotters. The mutations were not present in nongaited horses such as Thoroughbreds, Arabians, and Przewalski's horses. In the laboratory,

Anderson et al. (2012) blocked the normal function of the DMRT3 genes in mice (null mutants) and observed changes in locomotion and gaits similar to those in gaited horses. This research shows that the expression of certain specialized genes for emotion in vertebrates as different as mice and horses has remained remarkably conserved over evolution. One indicator of conservation of particular sequences of genetic code is that the code associated with emotional behavior in animals is also associated with brain disorders in humans (Castella et al., 2020; Chen et al., 2019).

Genetic differences interact with environment

Hausberger et al. (2004) highlight how individual horse personalities and temperaments interact with genetic, social, and nonsocial environmental factors. A battery of standardized behavioral tests was performed on 702 horses and ponies stabled at 103 different riding centers, training centers, and national stud and breeding farms in France. Only registered horses were used to enable accurate breed identification. Sex and age were well distributed in the horses (185 mares, 305 geldings, and 212 stallions). Behavioral tests were used to rate different facets of temperament, emotionality, and learning ability. An arena test was used to estimate emotionality. Each horse was released into a familiar arena, and its behavior was recorded for 10 minutes. Emotional reactions (whinnying, locomotion, vigilance) were used to evaluate the effects of *social separation* in the arena. This test differed from a standard open-field test used to measure fear because the arena was familiar to the horse. A novel object test was used to evaluate *nervousness*. An object resembling a cage with a ribbon attached to it was placed in the familiar arena. Each horse was released into the arena, and behavior was recorded (locomotion, gazes, approaches) for 5 minutes. A bridge test was used to estimate *fearfulness*. A foam mattress covered with checkered cloth (called a bridge) was placed in the familiar arena. Each horse was led to the bridge with a halter and the experimenter tried to encourage the horse to cross the bridge. For the *learning and memory* task, each horse learned how to open a wooden box with food inside, and subsequently it had to remember how to open the box 12 hours later. Given the fact that each test was designed to measure different facets of personality, the results of this study are extremely interesting. The results showed that genetic factors, such as sire or breed, influenced more neophobic reactions (fear of new things or experiences) such as the bridge test. On the other hand, environmental factors, such as type of work, played a more dominant role in learning abilities and reactions to social separation. Show and dressage horses exhibited higher emotional reactions to the social isolation test and the fear test (bridge) compared to horses used for other types of work. This is a notable finding, as a prior study also found a high level of stereotypies in dressage horses (McGreevey et al., 1995). Freymond et al. (2019) reported that horses that were more

sensitive to light touch were more likely to crib. Furthermore, the additive gene−environment effects uncovered are especially significant. In simple terms, additive effects mean that the expression of genes is influenced by differences in the environment. For example, the 98 breeding stallions tested in this study were stabled in two government facilities and kept under similar conditions (food, stall, little or no training for riding). The breeds were similar in both facilities, but environmental differences between the two facilities added to differences in the reactions to the bridge test (*fearfulness*), and the stallion's ability on the memorization test. The researchers suggest that varying handling practices and differences in caretaker behavior probably caused the differences in the behavior of the stallions in the two facilities.

Together, these two studies emphasize how far equine behavior science has come. The remaining sections cover additional studies on the following: the effects of early experience on behavior; the difficulties associated with finding easy ways to predict temperament; the relationship between hair whorls and behavior; and lateralization in the nervous system. The emphasis in each section will focus on interactions between the genetic and environmental effects on behavior.

The effects of early experience on behavior

Variability in emotional reactivity between animals is a phenotypic trait defined as temperament (Boissy, 1995). Horse breeders know that some foals are fearful and sensitive, while others are bold and confident. A variety of terms are used to describe temperamental differences in animals, but for simplicity the authors use the term "reactive" to describe animals that are flighty, nervous, or highly emotional. The term "low-reactive" is used to refer to individuals that exhibit low fear and low levels of emotional reactivity. Genetic variability in temperamental traits such as "reactive" or "low-reactive" are examples of the extremes within a normal distribution of temperament. In young foals, genetic influence and pre- and postnatal effects of the environment all contribute to individual differences.

Prenatal effects on behavior

A substantial body of evidence in animals and humans suggests that the gestational environment can impact fetal brain structure and function and increase long-term susceptibility to stress (Claessens et al., 2011; O'Connor et al., 2012). This can occur on its own, or in connection with genetic or postnatal factors. Environmental influence during fetal development is especially strong in the brain. Differentiation of major brain structures occurs during early gestation, creating more susceptibility to environmental conditions. Brain development involves a number of interactions with the environment, so even small changes from the normal developmental path during

fetal life can become progressively exaggerated over time, producing long-lasting or permanent changes (reviewed by Stolp et al., 2012). Recent work in humans shows that high levels of stress in the mother during pregnancy can affect brain function and behavior in her offspring, and that stress hormones are most likely transferred through the placenta (Glover, 2011). Although we are not aware of any studies examining the effects of prenatal stress in foals, we speculate that the effects of stress in the mare during gestation are similar to those shown in other animals (Weinstock, 2005).

Early postnatal effects on behavior induced by handling

A well-known book titled *Imprint Training of the Newborn Foal* (Miller, 1991) describes a handling procedure that consists of handling foals within 10 minutes of birth. "Imprint training" proposes to offer a singular opportunity to permanently mold a horse's personality. In this procedure, handling of the foals begins before the foals stand up, immediately following routine postnatal care of the umbilical cord. Foals are forcibly held in a lying position on the ground while the experimenter strokes them and exposes them to novel stimuli such as a white towel, a plastic bag, and a spray of water. Each stimulus is repeated until the foals remain immobile. These procedures last approximately 1 hour (Miller, 1991).

"Imprint training" has divided the breeding world into two distinct groups: passionate followers of the technique and those who are strongly opposed to this approach. Some equine behaviorists and veterinarians (including us) were never convinced that this procedure was a good idea—in theory or in practice. Since the publication of Miller's book, several studies have been conducted on the effectiveness of early intensive handling on foal behavior. There are conflicting results. A few of these studies closely followed the procedures of "imprint training" outlined by Miller, while other studies varied considerably. In 14 Arabian, 7 Quarter horse, and 2 Thoroughbred foals, Mal et al. (1994) and Mal and McCall (1996) looked at reactions to novelty, as well as individual manageability in minimally handled foals (routine and emergency veterinary care only), intermediately handled (10 minutes twice daily for one week), and extensively handled foals (same as the intermediate group and additionally once weekly for 10 minutes until weaning). During handling sessions, both sides of the foal's body from ears to tail head, including the belly and the legs to the knees or hocks, were rubbed. They found no difference between groups in manageability and response to novelty. Diehl et al. (2002) looked at mare−foal interactions in minimally handled foals (routine veterinary care) vs intensive (Miller method) handling of Standardbred foals. The mares were not restrained during the 4-hour postfoaling period, and no differences were observed in the behavior of the mares toward the handler in either group. However, mares of intensively handled foals spent less time eating and drinking, and more time

sniffing their foals. Using 15 foals of mixed breeds, Simpson (2002) performed an intensive handling protocol at between 2 and 8 hours following birth, and again once daily for 5 days. Reactions to stimuli similar to that used during the intensive handling procedure were measured at four months. Overall, the handled foals had more favorable scores for calmness and friendliness toward handlers, but there were no significant differences in compliance between handled and control foals. Williams et al. (2002) followed Miller's imprinting procedure closely, except they did not begin with the handled foals until the foals had stood and nursed. Forty-six Quarter horse foals and one Thoroughbred foal were handled at various times within the first 72 hours. Subsequent reactions to handling were measured at 1, 2, and 3 months old. They found no differences in handled compared to control foals. Lansade et al. (2004) followed a handling procedure close to the procedure used by Miller (1991) on 13 Welsh foals. The first handling session was performed between 1 and 6 hours following birth, the second at 24 hours, and the third at 36 hours following birth. The results showed that neonatal handling had short-term effects on manageability 2 days after the handling period (time to fit a halter, time to pick up feet, and leading on a halter). However, at 12 months old, there was no significant difference in manageability between the handled and control animals. In a review of these studies, Diehl et al. (2002) state that "there have been no consistent positive findings connected to early intensive handling with regard to compliance and long-term benefits in training or in reaction to novelty or potentially fearful situations."

To get a sense of the horse owners' opinions, we read online blog postings from horse owners with "imprinted" foals. Some of the words used to describe the behavior of the foals emphasized the bewildering and variable effects produced by intensive neonatal handling. These words included "trusting," "spoiled brat," "cooperative," "juvenile delinquent," "respectful," "pushy little monster," "drama queen," "lover," "friendly bugger," "tendency to go catatonic," and "potentially dangerous," to list a few (Horse Forum, 2012). It seems that the different responses of foals to early intensive handling are affected by several factors. Genetic factors influencing temperament may be one; another may be differences in the time the handling is performed; and last, differences in the way the handling procedures are performed may also influence responses.

Long-term effects of early intensive handling

Henry et al. (2009) conducted a groundbreaking study to monitor the behavior of "imprint-trained" foals in normal social groups with adults and peers from the early stages of development to adolescence. The results are both startling and disturbing. The subjects were 19 French Saddlebred mares and their foals (11 females, 8 males), all maintained under the same conditions

until birth. Delivery of the foals was not assisted and all foals received minimal care apart from the application of an antiseptic on their umbilical stumps. After birth, the foals were divided into one of two treatment groups: the control group, which included foals and mares left undisturbed (except for antiseptic application), and the experimental group, which included foals that had been handled following the "imprint training" guidelines (Miller, 1991). Apart from the early experimental handling procedure conducted on the handled foals, both handled and nonhandled (control) groups received only limited human contact necessary for routine procedures (mainly feeding and changes of pasture). They were kept under identical management, and the groups were mixed on the same pasture. The handled foals were later observed at four stages of life: during the early postnatal period, at 6 months old with their mothers, at weaning, and at 1 year old.

Early postnatal period

Handled foals stood up and suckled significantly later than control foals, and also displayed abnormal suckling activity. This suggested a delay caused by the handling procedure rather than a lowered capacity. However, handled foals displayed short-term disturbances not seen in the nonhandled foals, such as fast breathing and excessive trembling, indicating that the procedure significantly delayed these first two developmental stages. The abnormal suckling behaviors included excessive chewing and teat seeking directed "at the air" or at the handler and not toward their mother All of the handled foals struggled during handling (attempting to get up) before lying motionless with high muscle tone (Henry et al., 2009).

Six months old

Handled foals were observed on pasture with their mothers and the other mares and foals. Handled foals appeared more dependent on their mothers. They interacted more with their mothers than with the other foals and played less (especially social play). Handled foals also explored "new" objects in the paddocks less (such as an unfamiliar, motionless human) compared to control foals. Only three out of the nine handled foals approached the unfamiliar human, while all of the control foals approached and investigated the human. All of the observed differences between the handled foals and control foals involved social–emotional behaviors. Other behaviors (exploring, moving around, resting, self-grooming, etc.) did not differ from the control foals.

Seven months old (weaning)

Following separation from their mothers, both control and handled foals experienced similar levels of stress. Increased vocalizations and aggressiveness toward

each other were observed in both groups, which indicated that the social stress of weaning was similar in both control and handled foals. However, after the second day, emotional reactions of the control foals decreased, while high levels of vocalizations in the handled foals continued. Handled foals also engaged in less solitary or social play.

One year old

Both handled and control foals were separated and housed in same-sex groups. Most normal activities of the foals were not different. However, the experimental foals tended to withdraw socially, spend more time at greater distances from their peers, and exhibit more aggression. Control foals also displayed almost three times more friendly, positive gestures toward each other, while handled foals displayed more fighting, fleeing, or submissive behaviors. In this study, differences between the neonatal experiences of the foals revealed short-, medium-, and long-term effects of early experience on attachment and social competence later in life. As Henry et al. (2009) state, "These results underline the importance of this early stage for appropriate social development, despite later experience with peers."

All the studies reviewed here show that forcefully handling foals can have various effects on behavior, mostly negative. The negative effects of intensive handling may be more harmful in highly reactive breeds such as Arabians and Thoroughbreds. In any case, foals that are not handled may also present a problem. At some point, they need to be trained to accept handling for hoof care or health care. When these procedures become necessary, forcing foals may cause undue stress and influence the way they respond to handling in the future. A practical and easy way to teach foals to accept handling by people without making them fearful is needed.

A nonintrusive neonatal handling method

Over the last 20 years, the first author, Mark J. Deesing, has practiced a self-developed, nonintrusive neonatal handling procedure on his foals with some positive results. Immediately following birth and before the foal stands up, he applies an antiseptic to its umbilical stump. This is the only handling that the foal receives in the first few postnatal days. No more attention is given to the foal—instead, he concentrates his attention on the mare and shows the foal indifference. All of these procedures are conducted either on pasture or in a large paddock where the mare and foal are housed, and not confined in a stall. Each day following the birth of the foal, he spends a short period of time grooming the mare and feeding her carrots, but only if the mare approaches him willingly. As he cares for the mare, the foal becomes interested in the attention given to its mother, and walks around and watches from one side of the mare to the other. At first, the foal is hesitant to get too

close, but usually within a day or two, it moves closer and closer. Before long, the foal begins to sniff, then nuzzles him while he attends to the mare. When this happens, he continues to behave as if the foal is not there, showing complete indifference until he feels the foal is completely comfortable in his presence. After the foal approaches him several times and stands close by while he cares for the mare, he reaches out and touches the foal for the first time. The first touch is very brief. He gauges the reaction of the foal and then walks away. If the foal reacts to his touch, he knows not to touch it again until the foal has spent more time standing close by and investigating him. If the foal shows no reaction to his first touch, he knows that he can touch it more the next time. He learned the importance of not hurrying the first touch, and if the procedure is practiced this way, each day the foal allows more frequent and direct touching. Any forced contact results in a foal that avoids subsequent contact. Evidence for this is supported in a study by Henry et al. (2006). They found that forced stroking and handling of foals early in life did not improve the foal–human relationship. In a previous study, Henry et al. (2005) also found that softly brushing and feeding mares by hand had positive effects on foal behavior.

The first author practices his procedure in the same way on all his foals. Even so, differences are apparent in the time that it takes for the foals to touch him, or accept being touched by him. Some foals are fearful and take several days to get close and touch him, while others touch, smell, and nuzzle him on the very first day. In the beginning, he avoids touching them on the head or face. They seem to be more protective of their heads than other parts of their bodies. This is especially evident in reactive foals. To prevent any negative reactions, he is careful not to try to touch the head or face too quickly. Habituation to being touched on all parts of the body, including the head, takes more time in the reactive/high-fear foals, compared to the low-reactive/low-fear foals.

After the foal allows the second author to touch different parts of its body, he scratches and rubs it on the belly and the area around the top of the tail (Fig. 10.1). In his experience, foals seem to find this rewarding, and particularly like to be rubbed and scratched on these areas of the body. However, it may cause some foals to become pushy and begin to seek more and more of this attention. Attention seeking occurs sooner in less reactive foals and eventually occurs in more reactive foals. In any event, the moment a foal begins to push on him seeking attention, he gently pushes them away or walks away. This is similar to the way some mares behave toward their foal if she is busy grazing, or does not want the foal to nurse. If foals get too rambunctious or demanding, she might swish her tail, threaten, or walk away. The first author has found that handling foals this way teaches them to respect his space, and they also learn that seeking too much attention is unacceptable. The bond that forms is based on trust and respect.



FIGURE 10.1 Initial handling is performed while the mare and foal are not restrained or confined in a stall.

Factors affecting attachment to humans

A recent study conducted by Sankey et al. (2011) suggests that food is important in the human−foal attachment process, and that tactile contact is insufficient for bonding to occur. We disagree with this conclusion. The foals handled using the procedure developed by the first author actively seek tactile contact, which is not associated with another primary reinforcement such as food. No food reinforcement is used during the initial bonding process with the foal. In the Sankey et al. (2011) study, two groups of horses were reared either "under conventional domestic conditions," or in a forest reserve. No specific information was provided as to the horses' early contact with humans, or the early handling procedures used to habituate the horses to wearing halters. The forest-reared horses were "caught" and put with the stable-reared horses at about 10 months old and were 1−2 years old at the time of the experiment. In the methods sections of the paper, Sankey et al. (2011) state, "No additional contact with humans took place except for daily tethering for feeding."

We argue that the way tactile contact was perceived may have been influenced by early forced contact for haltering, even if force was kept to a minimum. Very few young horses (or none at all) freely accept being caught, handled, and fitted with halters without prior habituation to this procedure. In the Sankey, et al. (2011) experiment, the horses were tethered facing the wall and trained to remain immobile for 5−60 seconds in response to a vocal command "reste!" (French for "stay!"). Training was performed 5 minutes per day for 6 days. The two groups received either tactile contact (scratching the withers) or a food reward (a small piece of carrot) when they responded to the command. After 6 days, the experimenters performed a "motionless

human approach" test to assess the human–animal relationship. The results showed that the food reward group approached a stationary human sooner, and spent more time with the human. We do not argue the fact that food is a strong positive reinforcement. However, food motivation may override any reluctance to approach in the food reward group, compared to the group that only received scratching on the withers.

Horses raised by the first author actively seek tactile contact by humans. The second author, Temple Grandin, has noticed that they walk up to people on the pasture and present their belly or rear end for scratching, even toward unfamiliar people. What animals do not like a good belly rub? It is our hypothesis that *any* forced contact at an early age may possibly influence a horse's perception of humans as *potentially threatening*. In reactive/fearful animals, this perception may last a lifetime and be resistant to change. Without knowing the entire history a horse from the day it was born makes it impossible to know how a horse views people and will respond to any type of positive or negative reinforcement. Studying how horses view people, Fureix et al. (2009) found that perception of humans by horses may be based on experience. Handling and training can affect horses' memories of human actions either positively or negatively (Henry et al., 2006).

Mare behavior and human behavior influence foal behavior

Both mares and humans can influence foal behavior and their perception of humans during this early period. Henry et al. (2005) provided empirical evidence of this in a study of human–mare relationships and the behavior of their foals toward humans. Forty-one French Saddlebreds, Trotters, Arabian, and Anglo-Arab mares and foals were used in this study. The study included horses at two different sites. On site number 1, the mares and foals were kept in 3-m × 4-m box stalls. On site number 2, the mares and foals were also kept in 3-m × 4-m box stalls, but spent several hours each day on pasture with other mares and foals. Half of the mares at each site were brushed and fed by hand for the first 5 days of their foals' lives. The rest of the mares were not handled, and served as the control group. Gently brushing and hand-feeding mares for approximately 1 hour for 5 days following the birth of her foal positively influenced the foal's behavior toward humans. Foals spent longer periods close to the experimenter and initiated more sniffing, and licking contacts, whereas control foals appeared more fearful.

In this study, Henry et al. (2005) also observed the influence of mare behavior—protective or not protective—on the foal's behavior toward humans. Protective mares were described as nervous and exhibited behaviors such as always looking at their foals or positioning themselves between their foals and the human. Nonprotective mares were less nervous and did not try to block access to their foals. Foal behavior was recorded during tests conducted at 15, 30, and 35 days old. These observations were followed by an

approach-stroking test performed by familiar and unfamiliar persons at 11 and 13 months old. At all ages, the approach-stroking tests revealed that foals of protective mares remained further from the experimenter than foals from calm mares. Foals from calm mares also remained closer to the experimenter compared to controls and engaged in more sniffing and licking of the experimenter. In addition, foals of brushed and hand-fed mares exhibited reduced avoidance, reduced flight responses, and faster acceptance of saddle pads on their backs. Finally, handling of mares had effects on foals that were still measurable one year later. Both familiar people (experimenters) and unfamiliar people could rapidly approach and stroke the foals from the handled mares. The results strongly suggest that a mare's behavior can influence their foal's behavior toward humans. In this study, Henry et al. (2005) also found an interaction of breed and experience on human−mare relationships and the behavior of their foals toward humans. French Saddlebreds and Trotters were kept on site one, and Arab and Anglo-Arabs on site number two. On site one, French Saddlebreds and Trotter mares and foals were exposed to early handling. The foals were assisted to their feet following birth and moved to the mare to assist first suckling. (This is a common practice on breeding farms around the world.) In addition, their stable boxes were near the riding horses, and the mares and foals were involved in many visual contacts with humans. On site two, the Arabian and Anglo-Arab mares and foals received only minimal early contact by caretakers and were housed far from the riding horses. Overall contact with humans was very limited. Interestingly, breed differences in foal behavior were observed between the two sites. Arab and Anglo-Arab foals at site two were generally calmer and allowed human contact more readily than the foals at site one. This is another genetic−environment interaction. The more reactive Arab and Anglo-Arab foals benefited more from a *lack* of early and later human contact. Brushing and feeding mares by hand have a significant effect on foals' behavior toward humans. Henry et al. (2005) concluded that "this procedure is simple, takes little time, and can easily be applied to any dam−foal pair, as it is not intrusive and presents no risks of disrupting mare−foal bonds." In a subsequent study, Henry et al. (2007) re-tested the same foals at 6 months old and found that they remain sensitive to their mares' influences. The study also revealed high individual variability between the foals, suggesting a stronger effect of the foals' own behaviors at this age.

Serotonin genes and maternal behavior

The "protective" behavior of mares in the Henry et al. (2005) study represents a complex phenomenon. Naturally occurring variations in maternal care and protection are widespread in mammals, although researchers continue to differ in how much emphasis they place on genetic or environmental factors (McGue, 2010). In recent years, however, the pattern of care given

an offspring by its mother has been linked to variation in the serotonin transporter (5-HTT) gene (reviewed by Heiming and Sasher, 2010). Serotonin is an inhibitory neurotransmitter in the central nervous system that is linked to the regulation of mood and emotions. The 5-HTT polymorphism has two common forms: short and long. In human studies, the short 5-HTT gene form is related to increased stress sensitivity in young human females (Gotlieb et al., 2008), and in rats, maternal anxiety associated with the short form has also been found to influence infant temperament in the early postnatal period (Champagne and Curley, 2005). When mothers carrying a short form of the gene live in an environment that they perceive as dangerous or threatening, the effects of the short form are expressed, and their behavior can influence the behavior of their offspring. In a safe environment, the gene is not expressed. Consequently, Sachser et al. (2010) argue that the behavioral effects of maternal stress during the postnatal period in mice are not necessarily "pathological," but that the mothers may be adjusting the offspring to their environment in an adaptive way. Mothers may be preparing their offspring to live in a dangerous environment. Whether a mother carries a short form of the 5-HTT gene or not, pre- and postal effects of stress influence sensitivity to stress in offspring.

The effects of maternal stress or other postnatal environmental stressors are also influenced by genetically based individual differences in nervous system reactivity and temperament in foals. The research of Henry et al. (2009) on social behavioral development in "imprint trained" foals emphasizes this fact. Individual differences in behavior were observed in the intensively handled foals. Three out of nine foals approached and investigated an unfamiliar human, whereas almost all (9 out of 10) of the control foals left their mother and approached the human. One-third of the intensively handled foals approached the unfamiliar human, suggesting an effect of genetics on this response.

Training foals to accept handling

The neonatal handling procedure that the first author practices may help reduce stress in foals at weaning time by developing a strong human−foal relationship. The procedure begins very early and is continued throughout the time that the foal is with its dam. He handles the foal once or twice a week in the presence of the mare, and only for a few moments each time. All areas of the body are touched, including the legs and feet. By never using force and allowing the foal to initiate contact, the handling is perceived by the foal in a positive and rewarding manner. Teaching the foal to accept a halter begins during this time, but long before he actually halters the foal for the first time. As part of his weekly handling, each time he puts his arm over the foal's neck for a moment and gently pulls it against his body in a "hug-like" position. If the foal resists, he lets it go. Each time he does this, the

foal is more likely to accept it if no force is used the first time. During this entire process, the first author uses a calm, soothing voice and firm, but gentle, pressure to hold the foal. During the hug, he puts his free hand on the bridge of the nose, moving it up and down the face. He also places his free hand around and behind the ears. These movements are meant to simulate the movements of fitting a halter. After several short hugs over a few days, he begins to hold the foal for longer and longer periods. Soon, the foal completely accepts gentle restraint (hugs) without showing any signs of fear. Fear is evident if the foal suddenly raises its head, tightens its muscles, or widens its eyes. However, at some point the foal may try to pull away, not because of fear, but because it wants to do something else. When this happens, the first author begins to hold more firmly, for only a few seconds at first, and then for a few minutes at a time. If he sees any signs of fear, he lets it go. This allows the foal to predict what is happening and gives it a feeling that it has some control over the situation. Habituation to gentle restraint can occur quickly. The first author's method of positive reinforcement is similar to methods used by Phillips et al. (1998) to train flighty Bongo antelope (*Tragelaphus eurycerus*) to accept veterinary and husbandry procedures without chemical or manual restraint. In both these methods, the animal is habituated to a gradual increase in the intensity of the stimulus. Allowing an animal to have some control over stimulus presented in a predictable manner has profound effects on behavior in captive marmosets during routine handling and weighing (Bassett, 2003). In an excellent review, Bassett and Buchannan-Smith (2007) examined the link between predictability and control and made recommendations for simple and inexpensive modifications to husbandry routines that are easy to incorporate and can have a profound impact on the welfare of captive animals.

Wearing a halter and hoof handling

After the foal accepts being touched all over and held in the position for haltering, it is time to introduce the halter for the first time. He approaches the foal in the same manner as before, but he carries the halter, tucked into his belt so the foal does not see him carrying a novel object, which may cause fear. With the halter tucked into his belt, he gets the foal into the hug position, then slowly pulls the halter from his belt and gently slides it up the foal's nose. If the foal resists and pulls away, he lets go, remembering never to use force when introducing anything for the first time. If the foal does not resist, he slowly slides the halter up and down the nose a few times until the foal begins to show indifference. He then brings the halter fully up the nose and simulates the movements used to buckle the halter before actually buckling it for the first time. After the foal accepts this he buckles the halter and has the foal wear it for a few minutes, and then he takes it off. Every time he handles the foal, he follows the same consistent pattern: the first

approach, then hugging with an arm over the neck, then sliding the halter on. In most cases it takes only a few minutes each day for the foal to accept wearing a halter.

The next step is to teach the foal to lead with a halter and lead rope. Once the foal is habituated to handling and will accept a halter, he clips a lead rope onto the halter. The foal needs to see the lead rope and associate it with the halter, but learning to lead comes later. Holding the lead rope loosely, he stands next to the foal and starts to teach it to accept having its feet handled. Starting with a front leg, he slides a hand down the leg and tries to get the foal to flex the knee. If the foal refuses and pulls away, he lets it go. When the foal is settled down, he again approaches the front leg, slides a hand down the leg, and tries to flex the knee. If no force is used the first time, the foal does not associate having the leg handled as something to be afraid of and to subsequently avoid. Once the leg can be flexed and held for a moment, he moves on to the next. Each leg is handled the same way, never using force. Throughout this process, the foal is wearing a halter and the lead rope is held in one hand. Slowly, he begins to pull the foal's head slightly toward the side of the body on which he is standing, using the halter and lead rope to get the foal to watch as he rubs his hand down the leg. This is the first pressure that the foal feels from the halter and lead rope.

Subtle individual differences

A note here on individual differences: if a foal is of the reactive type, caution is required to avoid sudden movements during each of these steps to prevent causing a fear reaction. He keeps the lead rope loose to avoid any sudden pressure if the foal becomes frightened and tries to pull away. Habituation occurs quickly in less reactive foals, and less caution is required. All foals eventually habituate, but differences in reactivity and fearfulness are important to remember during halter fitting and picking up the feet. A basic principle is that each step of the training procedure must be introduced gradually to more reactive animals (Phillips et al., 1998). It is also important to remember that a time comes when foals are no longer fearful and may refuse to accept the hug, the halter, or having their legs and feet handled. This is a key point to remember, as motivation to resist handling can shift from fear to learned resistance that is not fear-motivated (Phillips et al., 1998). Once the foals overcome their fear, the motivation for self-gratification takes over, and resistance can be shaped by that attitude. Failing to recognize the motivational shift and allowing the foals to pull away is a mistake that, once made, can be difficult to correct. Motivational shifts can occur at any stage of handling, from the initial hug and hold stage, to the haltering stage, to the feet handling stage, and eventually to the learning to lead stage. A firm "NO" at the beginning of a motivational shift is an important reinforcement tool. This teaches the foals that they have to tolerate some things, even if

they do not want to. In the early training stages, failure to recognize motivational shifts and deal with them accordingly can cause subsequent resistance in the following stages of training. Learned resistance can stay with foals throughout their lives. However, if too much force is used to correct no-fear resistance and a foal starts to struggle, fear motivation can take over. In less fearful foals, resistance caused by no-fear motivation is stronger and more common. In fearful foals, resistance is primarily motivated by fear.

The next step is teaching the foal to lead and stand tied. After the foal used to wearing a halter and having a lead rope attached, the first author starts by leading the mare and encouraging the foal to follow. He keeps the lead rope loose on the foal so it can see it out front, but does not pull the foal with the rope. Once the foal follows, he begins to apply light pressure on the lead rope. In a short time, the foal can be led along with the mare: short distances at first, then farther and farther. Secondary reinforcement with a food reward begins at this stage. Using the "carrot and stick principle," he rewards the foal with a treat each time it moves forward willingly. He also uses a calm, reassuring voice to help to establish rapid habituation. Learning to lead and stand tied are both done in the presence of the mare. The first author teaches the foal to stand tied by leading both the mare and the foal to the hitching post, tying the mare, and wrapping the foal's lead rope over the post. In the beginning, he drapes the lead rope over the hitching post or wraps it around the post once and leaves it untied. This way, the foal learns to see the rope stretched out in front of it, but if it becomes fearful and pulls back, a fear of being tied can develop if is the foal is unable to get away. Staying close to the foal in the beginning is important. First experiences are critical to shaping future responses. A few minutes per week of standing (untied) alongside the mare is usually enough. Each time, the foal can be expected to stand for longer and longer periods. The first author never leaves a foal tied alone until after weaning when more time is spent on teaching the foal to accept being tied. To help the foal accept being tied alone, he starts moving away from the foal throughout this process—a short distance at first, then farther and farther away as time passes. The foal should not be expected to stand alone for any extended periods. Having a mare that calmly accepts handling and being tied also helps the foal accept handling.

More factors that affecting bonding with humans

As we discussed previously, Henry et al. (2005) found that mares can influence the behavior of their foals toward humans. A second hypothesis offered by Henry et al. (2005) is that foals can also influence the behavior of their mares toward humans. A person forcibly catching a foal and holding it while it struggles can upset the mare. The nonintrusive approach to foal handling practiced by the first author prevents the mare from becoming upset by seeing her foal in danger. As Henry et al. (2005) suggest, social facilitation is

involved in the human−mare−foal relationship. To demonstrate this principle, Henry et al. (2006) used three procedures to study early handling and human−foal relationships. In the first, foals were restrained and stroked all over their body. In the second, foals were restrained and taken to the dam's teat (first suckling assists). In the third, foals were exposed to a motionless person. The results found that the first two procedures had no effect on the later human−foal relationship, and the third slightly enhanced the foals' reactions to humans. Foals that were assisted during their first suckling avoided human approach and physical contact at 2 weeks old, and at 1 month old. This research supports the procedure practiced by the first author. His procedure begins with a human−mare bond, followed by a human−foal bond (only after the foal initiates first contact). The relationship is subsequently enhanced through social facilitation. To quote Henry et al. (2007), "It may be important that the young individual is an actor in the establishment of the relation, rather than a passive receiver of stimulations." In any event, foals' perceptions of humans develop from a very early stage. Whether a nonintrusive approach such as that practiced by the first author is used or not, several authors agree that gentle handling, stroking, halter fitting, and picking up the feet of young foals have more positive effects, at least in the short term than forcibly rubbing the foals' bodies (Heird et al., 1986; Jeziersky et al., 1999; Mal and McCall, 1996; and discussed in Henry et al., 2006). But the question of *when* to begin handling foals remains unanswered. Several authors argue the existence of a "sensitive" or "critical period" for learning to occur, and they suggest that the weaning period may be similar to those found in other animals at early ages. Lansade et al. (2004) addressed this by handling foals at two different life stages: immediately following weaning, and 21 days later. The results of this experiment found that haltering, petting, picking up feet, and leading foals immediately following weaning lowered their reactivity and made them easier to handle compared to nonhandled controls. The responses were also stronger in the early handled foals compared to the foals handled 21 days after weaning. Bateson (1979) suggested that stress induced by weaning causes greater sensitivity to external stimuli. Boivin and Braastad (1996) found more ease of handling in goats immediately following weaning and suggested that goats are susceptible to forming new social bonds during this period, and "that the human might serve as a surrogate mother." A complex interaction between genetic factors influencing temperament, pre- and postnatal influences, and other environmental factors affect the behavior of foals at weaning time. This is a stressful time. There are several psychological, physical, and nutritional stressors associated with weaning, along with other stressors, such as maternal deprivation and social isolation (reviewed by Waran et al., 2007). Forced weaning does not occur in nature. Since we are responsible for creating this adverse situation, we need to take the responsibility and make it as easy as possible for the foals to accept.

In summary, the effect of early experience on temperament, learning ability, and cognition is a complex phenomenon requiring further investigation, especially regarding the importance of the presence of stressors. In our opinion, people must make sure first experiences are positive no matter when handling begins. Forcibly catching young foals at any age may be detrimental to forming positive foal−human relationships. In addition, understanding foals' individual differences and motivational states, as well as appropriately handling resistance motivated by fear, or no-fear resistance, sets the stage for a relationship based on mutual trust and respect. This is similar to the social relationships that young foals develop with other horses. Inconsistencies in handling, especially at a young age, may cause conflicts between emotional and motivational states. Many of the assumed idiosyncrasies observed in foals (and mature horses) may in fact be pathological. For example, a common behavior in foals is to approach people and then quickly retreat, only to approach and retreat again. Are the foals curious but afraid (explained in Chapter 1) or are they exhibiting a conflict between a conditioned response of fear toward people and a similarly conditioned response motivated by the positive stimuli that people provide? Imagine for a moment the emotions of a foal that has been handled roughly, captured, haltered, and sometimes forced to stand to have its feet handled. These experiences may remain embedded in a young horse's memory and may be in conflict with other, more positive memories of people providing affection and food. Therefore, on the one hand, the foal fears people for the capture and forced handling. On the other hand, it likes people for the positive stimulus associated with them. Conflicts between emotional and motivational states are stressful and can compromise health, welfare, and responses later in life. The nonintrusive neonatal handling procedure practiced by the first author is easy and practical. To test its validity, studies similar to those conducted on intensively handling foals could be designed using this nonintrusive handling procedure and comparing the results with nonhandled controls. In the following sections, we discuss other aspects of foal and horse behavior that possibly influence this vulnerable period of life.

Genetics and temperament: origins and outcomes

Temperament is defined as differences in behavior between individuals that are consistently displayed when tested under similar situations (Hausberger et al., 2004; Kagan, 2005; Plomin and Daniels, 1997; Zentner and Shiner, 2012). Sackman and Houpt (2019) define a horse's temperament as "innate properties of the nervous system." The biological foundation is usually genetic but may be a result of prenatal events. Horses show considerable variation in temperament (e.g., fearfulness (Christensen et al., 2020), sociability, reactivity, etc.). Fearfulness is probably the most important factor influencing the suitability of horses for any type of work. Fearfulness is defined as the

general propensity of an individual to perceive and react in the same manner to a wide variety of potential threats (Boissy, 1998; Christensen, 2006). Abundant evidence demonstrates that past environmental factors and previous experience have a significant effect on fearfulness. Repeated exposure to stimulus early in life can modulate the development of subsequent fear reactions. Exposing a fearful young horse to an enriched environment and gentle handling procedures may reduce subsequent fear-related reactions. On the other hand, exposing a less fearful horse to a stressful environment and rough handling increase subsequent fear-related reactions. Early stimulation and later experience interact throughout the horse's life with the genetic background (Christensen, 2006). The ability to select a horse for a particular type of work, especially from a very young age, is an attractive idea for breeders, trainers, and horse enthusiasts. Behavior tests have been developed to identify temperament traits, but a factor that is usually overlooked is the biological basis of the traits being investigated. For example, by the time a horse is old enough to begin training for riding, it is difficult to detect the biological basis of temperament because the behaviors measured at this time could be a product of experience alone. Not all fearful horses inherit the biological basis of that trait. The origin of temperament has a genetic basis, but the outcome is influenced by environment and upbringing. Until recently, many temperament tests were designed to assess the fearfulness of a horse. Stomp et al. (2018, 2020) have discovered a behavioral indicator of positive emotions in horses. Snorting in horses is associated with positive experiences such as grazing on pasture or ridden with a loose rein.

A central question in studies of temperament is whether an individual's temperament traits remain consistent over time (Visser et al., 2001). In addition, the age at which temperament can be considered stable is still debatable (Seaman et al., 2002). The reliability of temperament tests of older horses is hindered by the fact that the background is usually unknown and the behavior is more influenced by previous experience. Thus previous experience may have more influence on responses measured in standardized behavioral tests than any underlying genetic influence. Previous experience affects older horses more than younger horses. In addition, previous experience may influence responses in a familiar setting, whereas response to novelty in an unfamiliar setting may better reflect horses' underlying reactivity (temperament). Standardized behavioral observations that depend on the experience and subjective judgment of handlers or caretakers are not always reliable. A handler's prior expectations of how horses should react may affect a horse's behavior, even if exact instructions are given on how to perform a test. Finally, standardized questionnaire surveys completed by knowledgeable handlers may also have limitations. However, comparing questionnaires with measures from standardized behavioral test assessments produce more valid and reliable results.

Finding easy and reliable ways to identify temperamental differences has two main advantages: the ability to predict behavior, and the means to select horses with suitable work-related characteristics. The following review is not exhaustive but is intended to show methods used by researchers to assess temperament traits. Some of these tests are practical and can be conducted by a lay person, but others are less practical. Suspending objects from a ceiling would be difficult to apply in typical riding centers or schools, where the ceiling is usually high. However, methods using novel objects such as an umbrella to test reaction to sudden novelty, or an unfamiliar arena to test emotional reactions to social isolation are more practical. The aim of temperament tests for horses is that interested people can perform the tests themselves and make use of the results.

Temperament tests

Behavioral measures

Visser et al. (2001) measured variation and consistency of horses of the same breed and age, reared under controlled housing and management conditions. Forty-one Dutch Warmblood horses were tested in two behavioral tests (novel object and bridge test) at 9, 10, 21, and 22 months old. The study identified two temperament traits (flightiness and sensitiveness), but did not show long-term consistency of traits. Seaman et al. (2002) recorded the behavior of 33 young horses in three behavioral tests (novel arena, human approach, novel object). Tests were repeated three times with an average of 9 days between trials. The responses of horses in a human-approach test and novel object test were similar but inconsistent: only the open-field test was consistent over time. The authors suggest responses in the open field may reflect a core factor of temperament. A questionnaire completed by the farm manager confirmed the results. Hausberger et al. (2004) used posture in 224 adult horses to evaluate factors involved in reactions to humans. The results showed breed differences but, more importantly, caretaker involvement revealed differences between the groups. One person cared for every horse in one school, suggesting the important influence of the caretaker on the well-being of the horses and their reactions to an unknown person. Sondergaard and Halekoh (2003) housed 40 young Danish Warmblood horses either alone or in groups of three after weaning. One-half of each group received handling three times per week for 10 minutes. At 6, 9, 12, 18, 21, and 24 months old, the study recorded behavioral observations in a human-approach test performed in the home environment. At 12 and 24 months old, the study recorded behavioral observations in a novel arena test followed by a human-approach test in a novel arena. Single-housed horses approached sooner and were more easily approached by a human in the home environment, but not in the novel environment. Single-housed

horses also expressed less restless behavior, more explorative behavior, and less vocalizations in the novel environment, but not in the home environment. Handled horses also reacted less to the novel environment. Results indicate that handling during the rearing period is a means of avoiding potential dangers associated with handling young horses in novel environments. Horses reared alone were reluctant to approach a person in the novel environment and moved around more in the novel arena. Visser et al. (2003) tested 41 young horses in four behavioral tests: a novel object test (lowering an umbrella from the ceiling), a handling test (crossing a bridge), an avoidance-learning test (avoiding a puff of compressed air), and a reward-learning test (choosing a manger to receive a food reward) during the first 2 years of life. Behaviors during each test were recorded, that is, locomotion, position relative to the novel object or bridge, posture of head and tail, latency times during the novel object or bridge test, and vocalizations. At the age of three, personality traits identified earlier (tail held high, latency time to touch a novel object, standing still in front of the bridge) correlate with early training in jumping performance. The study shows that a large part of jumping performance can be predicted by personality traits evaluated earlier in life. Momozawa et al. (2003) used a questionnaire survey to assess caretakers' impressions of temperament in 86 riding horses. The five-point questionnaire revealed three independent factors that they called "anxiety," "novelty seeking," and "understanding." A balloon reactivity test verified the reliability of the survey. Introducing each horse to balloons revolving in the center of an unfamiliar arena and recording heart rate for 5 minutes revealed that horses evaluated as "highly anxious" by caretakers had higher heart rates and defecated more during the balloon test. Also, horses evaluated as having problems with ordinary care or training had higher heart rates, and those evaluated by the survey as having a "long adaptation time to adapt to unfamiliar objects" were unwilling to touch the balloons. Results suggest that questionnaire surveys are a good way to assess temperament traits—especially those related to anxiety. Hausberger et al. (2004) used three behavior tests and a learning/memory test on a sample of 702 horses tested at 103 different sites. To establish precise genetic origin, the study used only registered horses. Study results show that the determination of behavior/temperament traits varies differentially with genetic influence (see our discussion of this study in the introduction to this chapter). Christensen et al. (2005) recorded heart rate and behavioral observations to stationary novel visual, olfactory, and auditory stimuli on 24 untrained stallions. The stallions were habituated to eat from a container placed in an arena. In the visual test, a traffic cone was placed in the arena near the food container; in the auditory test, a white noise was played while the horse ate from the container; and in the olfactory test, eucalyptus oil was applied to the inside of the food container. Results showed no difference between tests in locomotive activity, but heart rate increased during the visual and auditory tests. No increase in

heart rate occurred during the olfactory test, but the stallions ate more and became more vigilant to their surroundings. During the visual and auditory tests, more time was spent in being alert toward the stimulus. The heart rate responses correlated between tests and reflect a nondifferentiated activation of the sympathetic nervous system, while the behavioral responses were linked to the type of stimulus. McCall et al. (2006) recorded heart rate and behavioral observations in three tests: isolation, static novel stimulus, and traversing a novel stimulus in a runway. Forty horses performed all three tests on three different days. Both isolation and novel-stimulus tests produced valid measurements, but the novel-stimulus test was considered the more accurate evaluation of reactivity. In the isolation and novel-stimulus tests, walking and defecation frequency were the most precise behavioral measures. The authors suggest that using physiological data alone, combining physiological and behavioral measurements, or using more than one behavioral measurement in reactivity tests may better reflect the reactivity of the horses than a single behavioral measurement. Lloyd et al. (2008) assessed personality in 1223 horses of different breeds using a 25-item rating method previously shown to be reliable for personality assessment. The study identified breed differences and the breed differences shifted between personality traits. Anxiousness and excitability showed the most variation between breeds. Lansade et al. (2008) studied fearfulness in 66 Welsh ponies and 44 Anglo-Arabs. The tests included a novel object test, a novel arena test, and a surprise test. Results showed consistency in fearfulness between all three tests, as well as over time. Von Borstel et al. (2010) studied fear reactions in the following: untrained horses; well-trained dressage and show jumping horses; and horses with mixed genetic lines. An unfamiliar handler led horses into an arena containing a bucket of grain. When the horses began to eat from the bucket, a handler quickly pulled a weighted black plastic bag attached to a string across the arena. Results showed less intensive flight reactions in jumping horses compared with dressage horses or horses of mixed genetic lines. These findings suggest a genetic basis for less strong, though not less long-lasting, fear reactions in jumping horses compared to the dressage horses or the horses with mixed genetic lines.

Physiological measures

Schmidt et al. (2010) measured heart rate and stress hormone levels on seven Warmblood mares and six Warmblood stallions during a 12-week preliminary training program. Results showed that riding and training increased stress hormone levels. Mares had higher stress hormone levels than stallions, and mounting by a rider caused the most pronounced stress reaction. Leiner and Fendt (2011) measured heart rate and behavioral reactions to novel objects on 18 Warmblood stallions. The results show correlations between behavioral and physiological fear responses. Furthermore, after habituation

to an object occurs, the fear response to the object is reduced, whereas the fear response to another totally different novel object remains. Habituation to a stationary blue and white umbrella does not transfer to an orange tarp (Leiner and Fendt, 2011). Heart rate increases are a reliable measure of fear intensity in horses, although responses to the novel stimuli differed between individuals.

The purpose of this review was to show some of the methods used to study individual differences in temperament. Many of these tests are complicated and take a considerable amount of time to perform. In addition, a few are not easy to perform and difficult to standardize. For lay persons, it may be difficult to make decisions about a horse based on these results. However, the consistency of responses to sudden novelty, novel objects, and behavior in novel environments, all indicate a core dimension of temperament. As we discussed earlier, fearfulness is probably the most important factor influencing the suitability of horses for any type of work. In the next section, we discuss evidence collected by the first author that may provide an easy and practical method for assessing temperament that anyone can use.

The relationship between hair whorls and behavior

In early 1993, the first author approached me (Temple Grandin) in my office at Colorado State University to discuss some observations he had made while working as a farrier and horse trainer. The anecdotal evidence he collected suggested a possible relationship between hair whorls on horses' foreheads and temperament. I was of course skeptical; however, the story he told was compelling. All my years of working in the industry had taught me that people who work around cattle or horses daily often make valid observations that scientists working in the laboratory often miss. Before we met, Mark approached others in academia. Most said his ideas were too far-fetched to be worth consideration. We began by searching the literature for any evidence to support his observations. At the time, the only reference to horse hair whorls was a questionnaire survey of 1500 horses collected in 1965 by Tellington-Jones and Taylor (1995); however, the conclusions of the authors offered no statistical evidence.

What follows is the anecdotal evidence Mark discussed with me about hair whorls collected over a 15-year period and involving over 10,000 horses. He noticed hair whorls located high on the forehead predicted a flighty temperament. Whorls located below the center of the eyes predicted a calm temperament, and left or right side locations predicted handedness. He also noticed that environment and upbringing had an effect on whether the hair whorl could predict temperament and side preferences. He also noticed some unique and unusual behavior of horses with two side-by-side whorls. Following the anecdotal evidence presented by the first author, in the following section we discuss scientific evidence gathered by us over the last

20 years, as well as a review of more evidence compiled by other researchers around the world.

Personal observations

I (Mark J. Deesing) first heard about hair whorls in 1982, about 1 year after graduating from horseshoeing school. I was working full-time, shoeing horses and supplementing my income by breaking young horses to ride. That summer I was training a young Arabian filly, and I commented to her owner about how intelligent I thought her horse was. She said to me, "I know she's smart. My grandpa told me the high swirl her head meant she was smart." I had never heard of this, and I asked her to explain. Her grandfather said that Bedouin tribesmen 2000 years ago placed great significance on hair whorls, and believed the position of the whorls can predict how friendly or smart a horse was. (Hair whorls are located in an area between the eyes, single whorls can be located very high above the eyes, high above the eyes, between the eyes, low, and very low. Some whorls are on the right, others on the left (Fig. 10.2). Some horses have two whorls—one on both the right and left sides of the face.)

I thought it was a silly idea, but I was always curious to learn other people's ideas and opinions about horses. I wanted to understand horses better. My horseshoeing instructor taught me how to shoe horses, but not how to get horses to cooperate. Learning to understand and control horse behavior was a big part of my job. I did not understand why it is so hard to teach

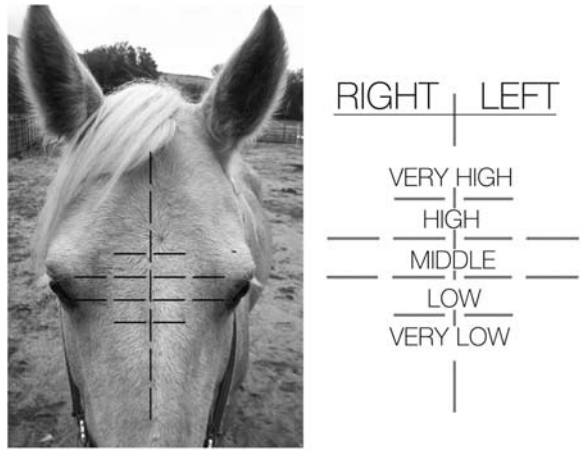


FIGURE 10.2 Hair whorls are located in an area between the eyes, single whorls can be located very high above the eyes, high above the eyes, between the eyes, low, and very low. Some whorls are on the right, others on the left (see Fig. 7.1.) Some horses have two whorls—one on both the right and left sides of the face.

horses something as simple as standing still for shoeing. In any event, what the woman told me was interesting, and I wondered if there was anything to it. I was shoeing 20 or more horses a week. It only took seconds to look at the whorl, and I dealt with horse behavior every day. The position a farrier works in while holding a foot off the ground is close-up and personal. Cooperation is necessary. I thought that if a relationship exists between hair whorls and behavior, I would know before too long.

I noticed right away that horses with high hair whorl(s) were more likely to resist shoeing. Horses with low whorls resisted less. At first, the relationship was not very strong, but strong enough to keep me interested. I began to value what I was seeing. Looking at the hair whorls on a horse before starting to work made my job a little safer. When I approached an excitable horse with a high whorl, I learned to avoid making quick movements or fighting with them if they resisted. Instead, I learned that a quiet and gentle approach worked better with excitable horses. Using this approach allowed me to finish the job without a fight, and without getting hurt. I was in a unique position to observe hair whorls in many horses. I was shoeing about 20 horses per week, 80 per month. In the first year alone I looked at more than 1000 horses. Even though I handled many horses more than once a year, the number of different horses I worked with far exceeded that of most professional horse trainers. I was learning about whorls from a variety of unfamiliar horses. I handled horses from all the major breeds, ranging in age from 6 months to 25 years. I still considered it just a curious phenomenon, but I found the relationship between whorls and behavior useful.

It was the behavior and hair whorls of my own three horses that started to convince me to take whorls seriously. Murphy was a 10-year-old Appaloosa gelding with a hair whorl well below the eyes. He was as calm as any horse I knew. I bought Murphy for my nieces and nephews to ride when they came to visit. Murphy had a carefree expression and an independent nature—he only worried about where his next meal was coming from. My other Appaloosa gelding, Dell, was just the opposite of Murphy. His whorl was well above the eyes. Dell was flighty and reactive. He would jump 10 feet if a mouse crossed the trail, and he was insecure and became very nervous when separated from the other horses. He was big and strong, but timid, reactive, and alert. He held his head high and oriented toward any unusual sight or sound. On the trail, he spotted deer on hillsides long before other horses. He would stop and point with his ears and eyes. My third horse was a race-bred Quarter Horse mare. Jenny had two high side-by-side whorls. Her behavior was similar to Dell's but less nervous: Dell's vigilance seemed motivated by fear, whereas Jenny's vigilance was more like a curious attention to her surroundings. She did not always need the company of other horses and was independent. Unlike Murphy's, carefree independence, Jenny had confident independence. As the dominant horse in the herd, she rarely exercised her authority by force: a slight pinning of her ears was all it took

to make other horses back off. When challenged, she let out a squeal that caused other horses to scatter. On the trail, she refused to follow another horse. Always the leader, she would throw a fit if I tried to make her follow.

Behavior differences in these horses were not subtle, neither were the hair whorls. When I saw similar hair whorls in other horses, I would ask the owners if their horses had similar behaviors. Clear patterns of behavior and hair whorls began to emerge. The patterns were still just trends, but it was around this time that I began to notice another, more consistent behavior related to hair whorls.

Hair whorls and side preferences

The awareness of side preferences in horses is common throughout the industry. The preferred side is easier to handle for shoeing, and horses have preferred directions when working in circles, or when ridden. The preference for one side during shoeing was so common that most horse owners would warn me about their horse's side preference before I started shoeing. I faced this problem every day. Before long, I noticed the hair whorl side (left or right) was often the difficult-to-shoe side. A left-side hair whorl predicted left-side resistance to handling for shoeing. A right-side whorl predicted right-side resistance, and double whorls predicted resistance on both sides. Hair whorl and side preference were more obvious than hair whorl height and reactivity. Side preference for shoeing was even obvious in horses with low whorls and a calm temperament. By questioning owners, I found the pattern common in all side preferences, but most common in horses behaving nervously. For example, I found that horses with a left-side hair whorl preferred the right side of a two-horse trailer, and horses with left-side hair whorls approached novel objects with a left-side bias, keeping the left eye on the object. All these behaviors were reversed in horses with right-side whorls, and always more obvious in nervous horses. Interestingly, horses with two side-by-side whorls showed less side preference in general. However, in horses with double whorls, I noticed that one whorl is usually higher than the other. The higher whorl typically shows the preferred side bias. These observations made my job easier, and much safer. For example, when I approached a fearful horse with a left-side whorl for shoeing, I learned to start the job on the horse's right side. This gave horses an opportunity to get comfortable with me before I handled the more difficult hair whorl side. I also found my observations useful when introducing young horses to the novelty of a saddle, mounting for the first time, and teaching them to rein to the right or left. Strong side preference seemed to be always motivated by fear. Horses showing strong side preferences often stood in defensive postures, holding their heads high, and showing the whites of their eyes. These are all signs of fear.

Hair whorls, temperament, side preference, and environmental interactions

After several years and several-thousand horses, I was finally a believer. Most horses with high whorls were reactive and fearful, but not all. I began to suspect the reason for this must be due to experience and upbringing. For example, I made most of my observations in the home environment, which could have masked fearfulness and reduced reactivity. And for most horses, horseshoeing is not a novel experience. I began to focus my interest on the effects of experience. Horses with high whorls and excitable temperaments often had excitable owners or owners who handled their horses roughly. Was it possible that reactivity and fearfulness of horses with high hair whorls was being masked by experience, or changed in young horses handled gently? Searching for answers led me to develop the nonintrusive neonatal handling practice described in the first section. I began to handle my foals all in the same way (regardless of hair whorl) and studied their reactions. It soon became clear that experience has a significant effect on behavior. If I handled foals with high hair whorls roughly, they resisted, and resistance continued in the future. On the other hand, gently handling foals with high whorls made them easier to handle in the future. The considerable variation in reactivity and fearfulness I observed in horses with high hair whorls was starting to make sense.

The paradox of double hair whorls

The puzzle pieces were falling into place—except for one. Horses with double hair whorls showed little variation. This presented a paradox. I never saw double whorls below the eyes. Double whorls above the eyes meant these horses should be similar to horses with single high whorls; however, this was not the case. Doubles were either very reactive and difficult to handle, or nonreactive and easy to handle. Very few individuals showed behavior between the two extremes. I did not know what to make of this. I also noticed differences in the way some horses resisted during shoeing or training. Horses with single high hair whorls reacted mainly with flight reactions, whereas horses with double whorls were more likely to fight, bite, or kick. On the other hand, when horses with double whorls showed good behavior, it was very good (Fig. 10.3). Many of the good ones also showed leadership and competitive traits, similar to my double-whorled mare, Jenny. I began to wonder about what caused them to be so different. To find an answer, the inheritance of hair whorls became the focus of my interest.

The hair whorls position is highly heritable

Over the years, I noticed hair whorl position was heritable. Yokomori et al. (2019) has not confirmed that hair whorl height on the horse's forehead is

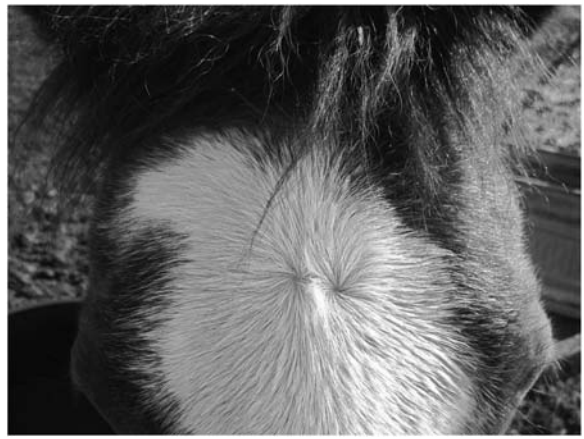


FIGURE 10.3 Double whorls. One whorl is usually higher.

highly heritable in Thoroughbred horses. For example, offspring of horses with high whorls almost always had a high whorl as well. Breeding a low whorl to a high whorl, offspring usually had a hair whorl in between the two but tending toward the high side. Inheritance of double whorls was harder to pin down. I got lucky one day while shoeing a mare. Her two offspring were standing at the fence, a yearling and a 3-year-old, both had double whorls. The mare had a high left side whorl. The owner said the sire belonged to the neighbor down the road. When I finished with the mare, I went down the road and asked to see the sire. His whorl was high, like the mare's, but his was on the right side. Surprised by the simplicity, I wondered if it was random. Determined to find out, it took nearly a year to confirm my first observation. After that, I went out of my way to look for other examples. Searching for examples was difficult, but over 2 or 3 years I found several following the same pattern. I noticed in all cases that whorls in the sire and dam were always high.

Many traits are conserved

After several years of observations and many discussions with owners about their horses, I discovered something I never expected. One afternoon I was shoeing a gelding with double whorls while talking to the owner about my observations. I explained that breeding a left-sided whorl (a "lefty") to a right-sided whorl (a "righty") is where the two whorls (ambidextrous) often come from. He looked at me with an odd expression and said, "My mother is right-handed, my father is left-handed, and I have two whorls on my head. My son also has two whorls and we are both ambidextrous." Frankly, I was shocked. I never considered something similar occurring in humans. I also

noticed the high heritability of double whorls in horses. I once saw double whorls in 15 out of 18 offspring from one stallion that had double whorls. I considered this more than just a coincidence but was no closer to understanding why double-whorled horses have such unique and unusual behavioral traits.

A Predisposition to pathology?

I finally found a clue in one of the most unlikely places, and it was not a horse that provided the clue. I was in a Laundromat one night washing my clothes and noticed a young boy there with his mother. He was obviously a "problem child," running around wildly, climbing on washers, and making noise. Each time his mother tried to settle him down, he was up and running again within minutes. When he happened to me, I noticed two pronounced whorls on the back of his scalp. Unable to contain my curiosity, I struck up a conversation with his mother. I asked the usual questions about handedness in her and her husband. She said she was right handed but her husband was ambidextrous. When she asked what it meant, I explained what I noticed in horses. I also explained the unusual behavior in horses with double whorls. With a disconcerted look on her face, she told me that 6 months earlier her son was well-behaved, and only recently started to change. When I asked what had happened to make him change, she mentioned being recently separated from her husband and going through a difficult divorce. They were sharing custody and she explained some inconsistencies in the way each of them handled their son. She tried to reason with her son if he was misbehaving, and her husband thought spankings were more appropriate. I thought to myself, "Many children go through the divorce of their parents and accept it well. What was different about him?" Maybe horses with two whorls are overreactive and more likely to exhibit behavior problems when faced with inconsistent handling or mistreatment? I decided it was time to find out. Soon after my experience with the boy and his mother, I traveled to Colorado to meet with Temple Grandin at Colorado State University. Her reputation as an objective, open-minded, scientist preceded her. If anyone could help me understand, she could. What follows in the next section is the scientific evidence that we collected over the next 20 years, and research conducted by others that supports my observations.

The science of hair whorls in humans

A review of the human pediatrics literature reveals that hair and brain form from the same fetal cell layer during gestation. At about the 10th week, the rapid development of the brain and the hemispheres occurs and the cranial vault rapidly enlarges to a domelike shape (Hall et al., 1989; Kiil, 1948; Smith and Gong, 1973). The expansion of skin over the dome-like outgrowth

corresponds to the position of the posterior parietal whorl—this much we know for sure. Interest by geneticists and neuroscientists in recent years focused on associations between hair whorl direction, language dominance, and handedness. The results of these studies are not mutually conclusive. Some investigators found a significant association between scalp-hair whorl direction and handedness (Beaton and Mellor, 2007; Klar, 2003; Friedman et al., 1952), others have questioned these findings (Jansen et al., 2007; Weber et al., 2006). Handedness has long been considered to result from both genetic and environmental factors (Corballis, 1997; Levy and Nagylaki, 1972; McManus, 1985). Klar (2003) was the first to report an association between handedness and scalp-hair whorl rotation and proposed the role of a single gene controlling both handedness and hair whorl orientation. Other investigators strongly argued against a common genetic basis of handedness or language lateralization with scalp-hair whorl direction (Jansen et al., 2007), and in a more recent report Klar (2009) found that handedness and scalp-hair whorl rotation is random and not genetically determined.

Finding a relationship between hair whorls and behavior in humans is complicated by the difficulty of determining hair rotation in people with long or curly hair. In addition, most studies only focus on whorl rotation (e.g., clockwise, counter-clockwise) and behavior—not whorl *placement* and behavior. Only Schmidt et al. (2008) looked at side *placement* and whorl *rotation*. The study revealed a significant association between whorls located on the center of the scalp and counter-clockwise rotation in nonright-handed children. None of these studies examined a possible association between handedness and language dominance in people with two or more hair whorls.

Hair whorls in cattle

In 1993 we noticed that hair whorl patterns in cattle were similar to those in horses. The second author (Temple Grandin) suggested that we aim our research toward cattle. We could study large numbers of cattle with similar genetics and experiences, a task more difficult with horses. Our first study revealed a significant association between the height of the whorl and behavioral agitation in cattle during restraint (Grandin et al., 1995; see Chapter 4: Genetics and behavior during handling, restraint, and herding). We studied behavior of 1500 cattle while restrained in a squeeze chute during routine handling for husbandry procedures. We rated the behavior on a scale from one to four (a cow rated "one" was calm, with no movement; a cow rated "four" was violently and continuously shaking the squeeze chute). Many of the cattle given a rating of four reared up in the squeeze chute, vocalized, and violently threw their heads during restraint. Cattle with hair whorls above the eyes were significantly more agitated during restraint when compared to cattle with middle or low hair whorls. The next study revealed an

association between positions of the hair whorls (e.g., left, right) and milking parlor side preference (handedness) in dairy cows (Tanner et al., 1994). For this study, we chose Holstein dairy cows because large numbers of cows could be monitored for milking parlor side preference with a computerized identification system. All cows were Holsteins and handling experiences were the same. Hair whorl positions and personal identification tag numbers were recorded on 1379 cows. Side preference data were collected for 90 milkings each. At the entrance to the parlor, cows had free choice to enter stalls on the left or right side. (Handlers only force cows to one side if all the stalls on the other side are full.) Electronic ID readers recorded the stall that each cow entered. Results showed that 45 cows with two hair whorls on the forehead were significantly less sided than the rest of the population. These two studies confirmed the anecdotal observations from the first author. Animals with high hair whorls are more reactive, and animals with two hair whorls show less side preference. Randle (1998) assessed the relationship between hair whorl position in 57 *Bos tauras* cattle and responses to humans. The results show the general response of cattle to a familiar human was not associated with hair whorl position, but the general response of cattle to an *unfamiliar* human was. Lanier et al. (1999) studied facial hair whorls and temperament in 1636 commercial cattle in the auction ring. Cattle with high hair whorls had higher temperament scores. Temperament scores also showed that Holsteins were calmer than beef cattle. Matson (2004) studied the relationship between hair whorl position and behavior in 146 crossbred heifers. One-half of the heifers were used as controls, and the other half were handled for 2 hours per week for 20 weeks. The handling consisted of walking through a chute, moving in a corral, sorting, and moving through a chute. Behavior of both control and handled heifers were scored in a squeeze chute, during isolation in a pen, and exit time from the isolation pen. Behavior measures were recorded in the beginning of the experiment, at week 10, and at 20 weeks. Results showed that handling treatment decreased in-chute behavior scores of heifers with facial hair whorl positions classified as medium or low, but not in heifers that exhibited a hair whorl high on their face. Broucek et al. (2007) investigated associations between hair whorl position and behavior in open-field and maze tests using Holstein heifers. The results did not find any significant differences in hair whorl positions in either test, suggesting an interaction between animals selected for a calm temperament and regular human contact. Holsteins in this study were a more homogenous group compared to the extensively raised (semi-wild) cattle used by Grandin et al. (1995). Broucek et al. (2007) found the differences among groups with high, middle, and low whorls were not significant, however, heifers with a whorl above the eyes showed more intensive movement in the open-field test and rapid learning and memory in the maze. More recently, Martins et al. (2009) found a high percentage of agitated cattle with hair whorls above the eyes compared to cattle with whorls between the eyes,

or cattle without whorls. Behavioral agitation was scored in a squeeze chute and the flight speed was measured after exiting the squeeze chute. Silveira et al. (2006) evaluated auction ring behavior of 1572 weaned calves with an average age of 210 days. Thirteen-hundred and seventeen calves were crosses between *Bos Indicus* (Brahman) and *Bos Taurus* (Angus, Red Angus, Hereford, and Charlolais), and 255 were pure *Bos Taurus*. The auction ring is similar to tests of social isolation in a novel arena but accompanied by sounds and movements of both the auctioneer and the public. Results showed that crossbred *Bos* Indicus × *Bos Taurus* cattle had higher hair whorls than pure *Bos Taurus* cattle, and behavioral agitation in the auction ring (escape attempts, restlessness, fast movements) was significantly higher in crossbred cattle compared to the purebred cattle. Florcke et al. (2012) studied variability in hair whorl patterns and differences in maternal protective behavior of beef cow−calf pairs 24 hours after calving. Results show that cows with a single high hair whorl or multiple hair whorls oriented toward an approaching vehicle at a further distance, compared to cows in other hair whorl groups. Also, cows with a high hair whorl vocalized at a further distance compared to cows in other groups. These results show that older cows and cows with high hair whorls are more vigilant of their surroundings. Shirley et al. (2006) studied heritability of hair whorls in Holstein cattle and reported three main conclusions: hair whorl height, asymmetry, and rotation are moderately to highly heritable polygenic traits; whorl height and asymmetry are the same traits regardless of whorl direction; and different genes are involved in the determination of each of these three characteristics.

Hair whorls in horses

In 1994 we conducted two preliminary observations on associations between hair whorl patterns and performance in horses. Hair whorl data was collected from 290 Thoroughbred horses during the racing season at Santa Anita Racetrack in Arcadia, California. Performance data was provided by The Daily Racing Form, a statistical service for bettors on horse racing in the United States. Statistical information on starts, wins, and money earnings were compared to hair whorl patterns. The horses were all registered Thoroughbreds, stabled at the track in 11 separate barns and managed by 26 different trainers, each with their own training, handling, and nutritional practices. No statistical correlations were found. In a second study, hair whorls were recorded on 133 Grand Prix jumping horses during the Masters at Spruce Meadows, Calgary, Alberta, Canada. Horses were various breeds from 15 of the world's top show jumping nations. Each horse was managed, handled, and trained with various training preferences. Performance data and rankings were provided by the Fédération Equestre Internationale, the international governing body for all Olympic equestrian disciplines. No significant correlations between hair whorl position and performance were found in this study either. However, some interesting trends

emerged, and of particular interest, two of the biggest money earners in each sample had double hair whorls. One of these was a horse named Big Ben. He was regarded as one of the greatest show jumping horses of all time. Inducted into the Canadian Sports Hall of Fame in 1999, he held two World Cup titles and two Masters Grand Prix wins. Big Ben was ridden by Ian Millar, one of the most successful competitors in the history of Canadian show jumping. Miller once described the gelding as "a skittish elephant," referring to his size and nervous temperament (Equestrian College Advisor, 2012). In the race horse sample, the horse with the most wins and money earnings was a 6-year-old gelding named Sir Beaufort. At the time, his earnings were $1.15 million, and in 1993 he was rated the number one older handicap horse in the nation. His trainer, Charles Whittingham, is one of the most acclaimed trainers in the United States racing history. Similar in temperament to Big Ben, Sir Beaufort had a slow start in his racing career because of his difficult nature. According to his owner, Victoria Calantoni, "He was a quirky horse. For a long time, he had trouble loading into the [starting] gate. They had to load him twice in the Santa Anita Handicap because his jockey broke a bridle. Besides that, he was always just a little headstrong. He doesn't like the whip a whole lot; if the jockey fought him, he just got worse, not better" (Meixel, 1993). These two horses illustrate the sometimes difficult nature of horses with two whorls observed by the first author. Horses with double or elongated whorls were also characterized by Tellington-Jones and Taylor (1995) as "unpredictable" or "over-reactive."

Increase of double whorls in racing and jumping horses

In the sample of racing and jumping horses, we found 16% (68 horses) with two whorls (Swinker et al., 1994). This percentage is considerably higher than the 4.4% (16 horses) in a sample of 362 Konik horses reported by Gorecka-Bruzda et al. (2006) and higher than the 10.9% double whorls (six horses) in the second sample of 55 Konik horses (Gorecka-Bruzda et al., 2007). Grandin et al. (1995) found 5% double whorls in 1500 cattle; Randle (1998) reported one double whorl out of 57 cows; Tanner et al. (1994) found 3.2% double whorls in 1379 dairy cows; Smith and Gong (1973) reported 7% double whorls in 200 normal children; and Lauterbach and Knight (1927) found 1.4% double whorls in 1003 normal children, and 6.5% double whorls in 751 children with developmental disabilities. It is intriguing that the percentage of double whorls in racing and jumping horses is nearly double the percentages found in all other samples cited in the literature.

Hair whorl height and reactivity in horses

Gorecka-Bruzda et al. (2007) published the first scientific evidence on the relationship between hair whorl position and reactivity in horses. The study involved two groups of young Konik horses reared under conventional

stable conditions (24 horses), or in a forest reserve under seminatural conditions (31 horses). The horses were grouped into four classifications based on their hair whorl position and/or shape: a single whorl high above the top of the eye line; a single whorl between the top and the bottom of the eye line; a single whorl below the bottom of the eye line; and four horses had an elongated or double whorl (Fig. 10.4). All of the horses were tested only once in three behavioral tests: being led away by a familiar handler (first moving away from the stable, then toward the stable); leg lifting; and a novel object test. In the novel object test, a familiar handler restrained each horse while another familiar handler approached and suddenly opened an umbrella from a distance of 5−6 m. Heart rate was monitored telemetrically during testing. The results revealed that horses with a high hair whorl were significantly more difficult to handle than horses with a medium or low hair whorl, but no significant differences were observed in cardiac reactivity. Horses with an elongated or double hair whorl were the most cautious of the groups when approaching a strange object. They took much longer than the single-whorled horses to approach and touch something new. The lack of a correlation between heart-rate measurements, startle responses, and hair whorl height in this study may be explained by the fact that Konik horses are known for a calm and gentle temperament (PetMd, 2012). This is similar to the lack of correlation between hair whorl height and temperament in

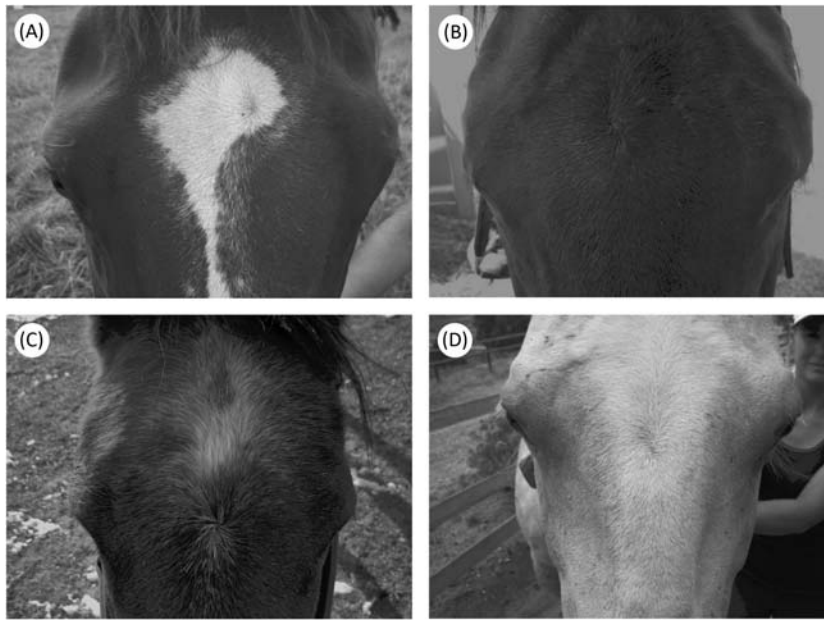


FIGURE 10.4 Classification of hair whorl positions on the forehead of the horse. (A) high right, (B) high left, (C) middle, and (D) low.

Holstein cattle reported by Broucek et al. (2007). (Holstein cattle are also known for their calm and gentle nature.) Gorecka-Bruzda et al. (2007) also found that hair whorl position in Konik horses is highly heritable. The estimates are similar to the heritability estimates of hair whorls in Holstein cattle (Shirley et al., 2006). It would be interesting to repeat the study of Gorecka-Bruzda et al. (2006) using breeds known to have highly reactive temperaments, such as Thoroughbreds or Arabians. It is clear that cattle producers, horse breeders, horse trainers, and horse owners could benefit from further studies of hair whorl position and rotation and its relationship to temperament. A new scientific study using genomic mapping of the horse provides evidence of a genetic effect which may govern the association between hair whorls and behavior (Lima et al., 2021).

Lateralization in the nervous system

Humans and animals have two types of laterality. One is motor laterality, the other is sensory laterality. The most common examples of motor laterality are right- and left-handedness in humans and side or limb preference in animals. Humans throw a ball or write better with either the right or left hand. Animals prefer to start walking using either the right or left foot, or the right or left paw to hold or manipulate objects. Sensory laterality refers to specialized tasks of both brain hemispheres. In most humans, the left hemisphere controls language, and the right hemisphere has a strong control of emotions. Although specialized functions of brain hemispheres were long considered unique to humans, increasing evidence points to behavioral asymmetry as a fundamental principle of organization in vertebrate behavior (Villortigara and Rogers, 2005; McGreevy et al., 2007; Rogers, Vallortiguara, 2021). At the most basic level, both sides of vertebrate brains show strong and consistent behavior patterns. In animals as diverse as chickens, fish, lizards, rodents, and humans, most *approach* behaviors (finding food and friends) are left hemisphere tasks, and most *avoidance* behaviors (responses to predators and novel stimuli) are right hemisphere tasks. When a horse attends to a visual stimulus EEG, readings indicate that the right hemisphere is activated (Rochais et al., 2018). Hausberger et al. (2019) state that brain lateralization may be a promising method for assessing learning and welfare. According to Rogers (2010), complimentary hemispheric specialization occurred during the course of evolution so that animals could search for food and remain vigilant for predators at the same time. Generally, the left hemisphere categorizes information and controls routine behavior, whereas the right hemisphere is responsible for responding to novel events, and coordinating rapid responses. Stressed or frightened animals rely on the predominant use of the right hemisphere (Rogers, 2010). Genes and environment influence lateralization, but the interaction between them is not always clear (Schaafsma et al., 2009). When animals such as dolphins or starlings become

curious and willing to explore, there is less lateralized behavior (Hausberger et al., 2021).

Laterality in horses

Horses show a preference to stand with one foot forward while grazing, to use either the right or left foreleg to initiate a walk or a trot, to roll on their right or left side, and to gallop on the right or left lead (McGreevy and Thompson, 2006; McGreevy and Rogers, 2005; Murphy et al., 2005; Wells and Blache, 2008; Williams and Norris, 2007). A recent study showed that a preference for a horse to use its right leg first when stepping from a stationary position may indicate that it has more positive emotions (Marr et al., 2018). Further study showed that horses that were successful problem solvers and able to learn how to turn a rod to operate a feeder were more likely to use their left eye (Esch et al., 2019). They were also more persistent and active compared to horses that failed to operate the feeder. When a sudden novel stimulus is presented, horses with a clockwise hair whorl will turn right and horses with a counter-clockwise hair whorl will turn left (Shivley et al., 2016). Training horses to work equally well in either direction is important in all equitation disciplines (Manroe, 1992; Maringer, 1983; Sivewright, 1986). Under saddle, horses tend to bend easily to one side and with more difficulty to the other side (Clayton, 1990; Murphy et al., 2005). Horses have long been considered asymmetrical (Steinbrecht, 1886), and many training exercises are aimed at straightening the horse.

Motor asymmetry

Whether the side preference is a result of genetic predisposition or if it results from training and use was unknown until recently. Grizmek (1968) looked into motor asymmetry and found that most horses begin to gallop with the left forelimb first and that they also turn left more easily. McGreevy and Thompson (2006) found differences in motor laterality between breeds of performance horses. The more temperamental Thoroughbreds showed a higher left foreleg preference during grazing when compared to Standardbred and Quarter Horses. Little or no side preference was shown in Quarter Horses. McGreevy and Rogers (2005) noted the relative position of the horses' forelegs every 60 seconds for 2 hours in Thoroughbreds grazing on pasture. Results show a population bias of standing with the left foreleg advanced. Forty-three out of 106 horses demonstrated a significant left leg preference, 10 horses demonstrated a significant right leg preference, and 53 horses were ambidextrous in their forelimb preference. In addition, motor laterality bias increased with age, suggesting maturation or influence of training. In contrast to the left foreleg bias observed by (McGreevy and Rogers, 2005; Murphy et al., 2005), found that 52% of horses preferred to start

walking or trotting with the right foreleg, while 40% preferred the left foreleg, and 7.5% showed no preference. The horses in this study were of mixed breeds (predominantly Thoroughbred × Irish Draught). The findings show strong sex-related *directions* of laterality, but not *degrees* of laterality. Male horses exhibited significantly more left-lateralized responses and female horses exhibited significantly more right-lateralized responses. Williams and Norris (2007) studied stride lead preferences in racing Arabian, Thoroughbred, and Quarter horse and found that 90% of ridden racehorses show a right lead stride bias. Wells and Blache (2008) studied both laterality in grazing stance and laterality in lunging circle tests in 15 ridden horses (5−20 years old), and 15 horses (1.5−3 years old) that had not been ridden. The breeds consisted of 60% Thoroughbred, 2% Arabian, 28% Warmblood, and 10% Riding Ponies.

The results showed that unridden horses did not have a grazing stance bias, but the ridden horses showed a right forelimb bias. The lunging test measured three indicators of bias; time spent in canter, correct lead in canter, and disunited canter. A disunited canter was when a horse began a canter on the correct lead, but then started to canter with an incorrect sequence of leads. In both the ridden and unridden horses, there was no correlation between grazing stance (relaxed) and any of the lunging indicators (challenged).

One possible reason for the conflicting results of motor bias is that different tests measure different characteristics of motor bias. Grazing stance, initiating a walk or trot, and rolling on the ground are biases exhibited while horses are in a relaxed state. Lunging and galloping biases are exhibited during a stressed (physical or psychological) state. Different conclusions about motor bias may be explained by test conditions, breed and temperamental characteristics, and selective pressures for a particular type of work. McGreevy and Thompson (2006) suggest that some breeds may have inadvertently been selected for laterality, or ambilaterality, due to the nature of the work they perform. It is not known if this is possible in horses, but selective breeding for laterality is routinely done in laboratory mice (Collins, 1975).

Sensory laterality

Sensory laterality refers to the preferred use of one eye, ear, or nostril over the other under particular circumstances. Science attributes sensory laterality to the processing of different types of information in one or other of the two brain hemispheres (Andrew, 2002; Rogers, 2009, 2010; Villortigara and Rogers, 2005). Rogers (2009) and Proops and McComb (2012) suggest that sensory laterality evolved before motor laterality. Evidence to support this is found in fish without limbs that show lateralized eye preferences to view certain scenes (Bisazza et al., 1997), and prey catching on their right side

(Takeuchi and Hori, 2008). Scientists that once believed that hemispheric asymmetry is unique in humans now embrace the evidence for lateralization in a wide variety of species (summarized by Rogers, 2009, 2010; Villortigara and Rogers, 2005).

Until recently, there was no evidence of sensory laterality in horses. Heird and Deesing (1998) were the first to discuss sensory laterality and noticed when horses are suddenly startled or frightened by novel stimulus they focus one eye on the object and make lateral body movements around it. Over the last decade, sensory laterality in horses has attracted considerable attention (Austin and Rogers, 2007; Larose et al., 2006; McGreevy and Rogers, 2005; Williams and Norris, 2007). Austin and Rogers (2007) and Murphy et al. (2005) recorded flight and escape-turning responses in 30 horses of various breeds exposed to a novel object and recorded greater fear responses to fear-inducing stimuli presented to horses' left eyes, and therefore the brains' right hemispheres. In this study, an experimenter opened an umbrella 5 m away from the horse, first to the horse's left side and then to the right side or vice versa. Horses tested first on the left side showed greater reactivity and flight responses for the left-side approach, whereas horses tested first on the right side displayed less reactivity and shorter flight distance when subsequently approached on the left side. The evidence suggests that stimuli first presented to the right side allowed the horses to learn that the stimulus posed no threat. This information then transferred to the right hemisphere of the horses' brains, leading to a lessened reaction when approached with the umbrella from the left side. Larose et al. (2006) studied visual asymmetry during exposure to novel objects in Trotters and French Saddlebreds. The temperaments of the two breeds were similar, but expressions of visual laterality differed between individual horses. Horses with higher emotional scores assessed during behavior tests glanced at a novel object with the left eye (right hemisphere) more than the right eye (left hemisphere). This evidence shows that laterality is stronger in more reactive (fearful) animals. In addition, stimuli that animals fear the most, such as sudden rapid moving objects, or objects that animals have been conditioned to fear influence laterality. De Boyer des Roches et al. (2008) found that emotionally laden objects influence lateral responses. Horses showed a slight tendency to use the left eye (right hemisphere) to explore objects with negative emotional value (a veterinarian's white shirt) more than objects with neutral emotional value (a plastic cone) or positive value (a feed bucket). The age of a horse can also influence laterality. Sankey et al. (2011) found that yearling horses approached on the left side showed more escape and threat (avoidance) behaviors, as opposed to more positive (approach) behaviors when approached from the right side. Two-year-old horses, on the other hand, reacted positively to approach and contact from both sides. These results show the influence of work and training on laterality in horses. It has been argued by some that asymmetries in horses are caused by humans

traditionally handling horses on the left side. In Trotter horses that are, to an extent, handled on both sides, Larose et al. (2006) found no association between eye preference and reactivity. However, field studies of feral horses by Austin and Rogers (2007) show that lateralization is a characteristic of horses as a species that is not caused by human handling. Observations revealed a left-side bias of reactivity (heads held high and looks to the left or right caused by the presence of humans), a left-side bias in vigilance (raising the head from a grazing position and turning the head to attend to stimuli), and a left-eye bias during aggressive exchanges (eye used by stallion to look at its opponent). The left-eye bias was stronger during attacks than during threats. There was no observed population bias of foreleg preference in grazing stance in feral horses, compared to population biases in domestic horses. The absence of population-level forelimb preference in feral horses suggests that training and work influence forelimb preference in domestic horses (Austin and Rogers, 2007), and that limb preference does not indicate brain lateralization, but may better reflect a horse's attention to its environment.

Auditory and olfactory laterality

Basile et al. (2009) reported the first evidence of socially dependent auditory laterality in horses. Head and ear orientation responses to whinnies recorded from both familiar and strange horses showed that horses turn their heads to the right (right ear orientation) toward recorded whinnies from familiar horses, whereas no bias was found during calls from unfamiliar horses.

McGreevy and Rogers (2005) investigated olfactory laterality in Thoroughbred horses (32 males, 125 females) less than 4 years old. Horses were presented with olfactory stimuli (stallion feces) and a right nostril preference was found in younger, but not in the older horses.

These studies clearly show that the right and left sides of horses' brains are specialized to handle information in different ways and to control different classes of behavior. In addition, varying strengths of lateralization have been found between breeds, and in individuals within a breed (De Boyer des Roches et al., 2008; McGreevy and Rogers, 2005; McGreevy and Thompson, 2006; Sankey et al., 2011). Specialization of hemispheric control of behavior during stressful and nonstressful conditions is obvious and has important implications for horse welfare (Rogers, 2010). In a relaxed state, most horses use the right eye (left hemisphere) to process information. In a stressed state, the dominant use of the left eye (right hemisphere) may compromise welfare. This is especially important considering that the right hemisphere also controls endocrine function, heart rate, and blood pressure (Conrad et al., 2011; Wittling and Pfluger, 1990). Recent data also suggest that the right and left cerebral hemispheres differ in their ability to regulate autonomic processes, and direct unilateral stimulation of the brain provokes side-dependent endocrine, immune, and other visceral reactions. The study of laterality and

fearfulness can be used as a new approach to investigate the processes that underlie the interindividual variability of the vulnerability to stress and stress-related diseases.

Hemispheric dominance

Population asymmetries, such as right-handedness in humans, show consistent directions. Reversed or absent asymmetries occur in some individuals (Corballis, 2009). Ghirlanda et al. (2009) note population-level handedness reversals ranging from about 10% in humans to about 35% in chimpanzees (Corballis, 2009). In 326 normal humans, Knecht et al. (2000) measured lateralization directly by functional transcranial Doppler sonography and found the incidence of right-hemisphere language ranged from 4% in strong right-handers to 15% in ambidextrous individuals and 27% in strong left-handers. Knecht et al. (2000) suggest that these results demonstrate that the relationship between handedness and language dominance is not an artifact of cerebral pathology but a natural phenomenon. Discussing reversals in horses interacting with humans, Farmer et al. (2010) write, "Intriguingly, the right-eyed horses in both groups, although only a very small sample, showed the same trend with regard to the right eye, that is to say they showed stronger lateralization under the stranger (response to an unfamiliar human) condition."

In the studies reviewed in the previous section, the few horses in each sample showing a reverse of left-eye (right hemisphere) processing of emotional information have received little attention. It is interesting to speculate that reversed hemispheric dominance might reflect a distinct cognitive disposition or bias. An association between cognitive bias, handedness in humans, and limb preference in animals supports this view (Rogers, 2010). Cognitive bias refers to the particular way the brain perceives, forms memories, and makes judgments. Denny (2009) found that left-handed people report more depression symptoms than right-handed people. In chimpanzees, Hopkins and Bennett (1994) found that right-handed chimps interact more with novel objects than left-handed individuals. More evidence suggests that preferred use of the left paw (and right hemisphere) is characteristic of more fearful marmosets (Cameron and Rogers, 1999). Rogers (2009) also found that left-handed marmosets are more likely to have higher levels of the stress hormone cortisol when compared with right-handed individuals. Rogers (2010) also suggests that right-handed marmosets are active, and left-handers are reactive. In marmosets, handedness predicts a tendency to adopt a positive or negative cognitive bias.

In horses, no studies exist that specifically address differences in left-eye system *vs* right-eye system dominance and cognitive bias. It may be possible that right-hemisphere-dominant and left-hemisphere-dominant horses respond differently in similar circumstances. Anecdotal evidence collected by the first

author suggests that horses with *high* left- or *high* right-side hair whorls respond differently to either stationary or sudden novelty. Horses with *high* right-side whorls display higher levels of arousal such as prancing, vigilance, whinnying, snorting, and tail raising, compared to horses with *high* left-side whorls. To illustrate this point, the first author has two Warmblood mares; Bobbi has a *high* left-side hair whorl, and Sam has a *high* right-side hair whorl. The mares are both 6 years old and share the same sire, but different mothers. The first author bred, raised, and trained both mares himself. Sam and Bobbi are both reactive to sudden novelty and social separation; however, Sam (right-side whorl) vocalizes when separated and makes more attempts to return to the herd. Sam also nickers and whinnies more than Bobbi at feeding time. Emotional responses to positive and negative stimuli between the two are obvious. Recently, we tested both mares on flight and escape-turning responses using the method described by Austin and Rogers (2007). Placing an oat bucket in the center of a large paddock, the first author released the mares one at a time and allowed them to eat from the bucket for 1−2 minutes. He positioned himself in front of each horse to make sure they were looking at him using both eyes, then quickly walked toward each horse while suddenly opening an umbrella. Sam quickly turned and escaped to the left, Bobbi quickly turned and escaped to the right. There was no difference in the immediate response, and both horses ran about 10 m before stopping and turning to look back. After opening the umbrella in front of each horse, the first author moved out of the paddock and noted the time it took for each horse to return to the feed bucket. Bobbi returned to the bucket and resumed feeding within 3 minutes. After 10 minutes, Sam had not returned to feed, and for roughly 5 minutes after the umbrella, she snorted, displayed a raised tail, and pranced. Horses snort when startled, in pain, or when frightened (McGreevy, 2004). Prancing, vigilance, and a raised tail are attributed to high levels of emotional arousal (Waring, 1983; Wolff et al., 1997). None of these signs of emotional arousal were shown by Bobbi, and in the following days behavior toward the first author was unchanged. However, Sam was reluctant to approach the first author for several days following this experience. This suggests a strong negative memory of the experience associated with the first author. The escape turning preference of each horse is also the same as their turning preference when lunged in a circle, but only when the horses are nervous. For example, when they have not been lunged for weeks, the first time lunging after time off they get slightly nervous and directional preference is expressed. However, after a few minutes on the lunge line, they relax and directional preference disappears.

This preliminary experiment is merely anecdotal, but a controlled experiment conducted using right- and left-side hair whorl horses may provide evidence of cognitive bias. The tests most likely to cause emotional reactions are exposure to sudden novelty or social isolation in a novel arena. It would

be interesting to study heart-rate variables and stress hormone levels following response to novelty in horses with right- or left-side hair whorls.

Hair whorl patterns and lateralization

Murphy and Arkin (2008) found a significant association between hair whorl rotation (direction) and motor asymmetry in horses. The study recorded rotation of single hair whorls in 219 horses (males = 125, females = 94), between 4 and 6 years old. The distribution of whorl patterns was 114 counter-clockwise, 82 clockwise, and 23 radial (no rotation). Experienced trainers rode and jumped each horse to determine motor laterality. Results showed that right-lateralized horses had significantly more clockwise hair whorls, and left-lateralized horses had more counter-clockwise hair whorls. More recently, Ovando (2010) found no association between hair whorl rotation and motor asymmetry. The study recorded rotation of single whorls in 50 Thoroughbred foals (males = 29, females = 21) all under 1 year old. The distribution of whorl patterns was 16 counter-clockwise, 10 clockwise, and 24 radial (no rotation). The foals were tested 50 times each using the first step described by Murphy et al. (2005). Results showed no association between hair whorl rotation and asymmetry but did find that males were more left-lateralized, and foals with radial whorls were more balanced (ambidextrous). These results contradict those obtained by Murphy et al. (2005), and the difference in results may be caused by the difference in the age groups. The yearling foals studied by Ovando (2010) were all the same age and received halter training but otherwise minimal contact with humans. The horses studied by Murphy et al. (2005) were all trained to ride. This finding is in agreement with McGreevy and Rogers (2005) who found that horses under 2 years old were less lateralized, compared with a higher percentage of left-lateralized horses over 2 years old. It may be that the "motor" laterality observed in ridden horses is actually motor asymmetry influenced by sensory laterality influenced by stress induced by training and riding. It is recognized that the amount of psychological stress (fear) that an animal encounters determines the degree of response of the hypothalamic–pituitary–adrenal axis (Cayado et al., 2006). Blood samples collected in Dressage and Jumping horses at their familiar home environment were compared to samples taken before exercise, at the entrance to a schooling area, and postperformance over jumps or a dressage course. Plasma cortisol and plasma ACTH were highest in the least experienced horses, high in horses with intermediate experience, and slightly lower in the most experienced horses (Cayado et al., 2006). The fear responses in the most experienced horses were attenuated, but still evident.

The inconclusive results of hair whorl direction in horses and asymmetry are similar to the inconclusive results of studies of hair whorl rotation and handedness in humans (Beaton and Mellor, 2007; Jansen et al., 2007; Klar,

2003; Weber et al., 2006). In addition, all these studies only focus on whorl rotation (e.g., clockwise, counter-clockwise) and laterality—not whorl *placement* and laterality. Anecdotal evidence presented by the first author suggests that hair whorl position (e.g., left or right of midline) is a more reliable predictor of laterality in horses. Support for this anecdotal evidence was recently reported by Tomkins et al. (2012) in dogs. Specifically, the location of a whorl on the left side of a dog's head and neck was associated with a right visual bias, and a right visual bias was also probable if the hair whorl on the ventral mandibular (back of the bottom jaw) was present in a counter-clockwise direction. The study assessed all three measures of laterality (hair whorl, motor, and sensory) in Labrador retrievers, golden retrievers, and Labrador−golden retriever crosses. The dogs were prospective guide dogs for the blind provided by Guide Dogs NSW/ACT. Dogs' performances on the Kong test, the First-Stepping Test, and the Sensory Jump Test determined motor and sensory laterality. Several associations emerged between hair whorls and motor and sensory laterality. The authors suggest that hair whorls may provide a quick and efficient means of determining suitability in prospective guide dogs because a need to work on the left side of trainers often disqualifies dogs that are less flexible when turning right. It also takes a significant amount of time to train guide dogs. The dogs must learn several complex tasks, and it is important to find a quick and efficient means of discovering traits in the early stages of training that decide whether a dog will be a successful guide dog.

Clearly, further research is needed to determine associations between hair whorl direction and laterality in horses. Lateralization is important because it is related to locomotion and subsequent performance during training. Determining lateral trends at a glance could be very useful for designing individual training programs.

Chronic asymmetry

In his experiences as a horse trainer, the first author has faced several one-sided horses. In some horses, the turning preference was so strong that any attempt to change it caused the horse to prance, throw its head, or rear. Any use of force to change a preference was likely to cause a panic-like response. To our knowledge, no scientific research into the cause of extreme laterality in horses exists; however, various explanations of extreme one-sidedness exist in the popular horse-training literature. The most often-cited reasons are strong motor asymmetry or pain. Lateralized behavior is undesirable in various equitation disciplines, and persistent lateralized behavior has not been thoroughly investigated. Scientists and industry professionals rarely discuss fear as a possible cause of extreme one-sidedness. All the same, evidence of brain asymmetries previously discussed points to many lateral behaviors associated with fear responses. Fearfulness may be more common

in horses than is generally assumed. Horses do not require many fearful experiences for fear behavior to be habitually expressed, and many chronic fear behaviors in animals and humans can be traced back to a single event (Lindsay, 2000). One-trial-learning of fearful responses is documented in horses (Kiley-Worthington, 1987). Fear responses are learned at the subcortical level involving the amygdala and hippocampus (LeDoux, 1998), with direct projections to the motor cortex. Lateral behavior and hair whorl side-position has been demonstrated in cattle (Tanner et al., 1994), and fear responses are associated with hair whorl height-position in cattle (Grandin et al., 1995; Silveira et al., 2006), and horses (Gorecka-Bruzda et al., 2007). Although hair whorl side-position and lateral fear responses have yet to be demonstrated in horses, anecdotal evidence collected by the first author suggests an association between lateralized fear responses and the side position of hair whorls in such things as handling feet, lunging in circles, riding, reining, trailer loading, and jumping. Left-side hair whorls predict left-side resistance to horseshoeing and predict a preferred rightward direction of travel when working in circles. In many cases, these lateral behaviors are associated with obvious fear responses; in others, the fear responses may become attenuated over time (Cayado et al., 2006), and can easily be mistaken for motor asymmetry or disobedience. For example, young horses trained in a round pen on a lunge line often travel better in either a clockwise or counter-clockwise direction. If horses have a left-side hair whorl, the preferred direction is clockwise. In the counter-clockwise direction, the horse makes smaller circles and tries to turn its head to the outside. The head is often held high, the speed of travel is increased, and the tail is held high. These are all signs of emotional arousal (McGreevy, 2004; Visser et al., 2001; Waring, 1983; Wolff et al., 1997). Older horses show the same signs of arousal but to a lesser degree. As we discussed earlier, horses with left-side whorls resist horseshoeing on the left side. The resistance may involve attempts to flee, or refusal to allow a leg to be touched. In other instances, the resistance may be subtle and horses stand and tolerate the leg being lifted, but lean on the farrier or try repeatedly to pull the foot and leg away. These are not clear and obvious signs of fear, but may in fact be attenuated fear responses. Leaning on a farrier may be a defense reaction motivated by fear, and more evident on one side. The "assumed" motor asymmetries shown by horses in ridden work (Murphy and Arkin, 2008) may in fact be attenuated lateralized fear responses (Cayado et al., 2006). In the early stages of training for riding, fear may motivate horses to prefer to work in a rightward or leftward direction. Horses with left-eye (right hemisphere) control of fearful stimulus may be less fearful when working in a rightward direction. This direction allows the horse to keep the left eye to the outside of a circle where it feels more confident to defend itself against potential threats. Strong one-sidedness motivated by fear in young horses during the early stages of training may be attenuated with experience and mistaken for motor asymmetry.

Research in humans and animals shows that trauma experienced early in life, or at any time in life can cause shifts in cognitive bias toward the right hemisphere leading to prolonged anxiety-like behavior (Chamberlain and Sahakian, 2007; Mathews and McLeod, 2005). Adamec et al. (2005) exposed rats to a cat and found a shift to the right hemisphere, and subsequent changes in neural activity and neurotransmitter levels lasting up to 12 days after this stressful event.

Fear stress is highly aversive and subjecting animals to extreme fear stress is very detrimental to welfare (Grandin, 1997).

Experience-dependent lateralized learning

In a previous section, the first author discussed his approach to horses that are difficult to shoe on one side or the other. He found that switching sides and shoeing the "less difficult" side first was helpful. When he finished the less difficult side, horses were often more cooperative when he returned to the difficult side. This may be an example of experience-dependent lateralized learning. Information processed and learned in one hemisphere of the brain transfers to the opposite hemisphere in an experience-dependent manner. Studies report interhemispheric transfer of memory in species as diverse as fish, amphibians, reptiles, birds, and mammals (Robbins and Rogers, 2006; Villortigara and Rogers, 2005). In cattle, Robbins and Phillips (2010) report the first example of experience-dependent lateralized learning in a mammal. In herd-splitting experiments conducted on dairy and beef cattle, a single experimenter walked repeatedly through herds, either empty-handed or while carrying various novel objects. At first, the cattle viewed the experimenter using their left eyes (right hemisphere). After habituating to the experimenter carrying various objects, the cattle then switched to viewing the experimenter with the right eye (left hemisphere). This directional shift in viewing preference was experience-dependent. Robbins and Phillips (2010) suggest that the reversal of preference from the left eye (when the stimuli are novel) to the right eye (when the stimuli become familiar) "frees up" the left eye to simultaneously watch for new threats.

Switching sides

There is no known experimental evidence of experience-dependent lateralized learning in horses. However, it may be safe to assume that this well-conserved pattern of lateralized learning exists in all mammals (Robbins and Phillips, 2010). During horse training, the first author switches sides when a horse shows a lateralized response. He also uses hair whorl positions (i.e., left or right side of the forehead) to predict a lateralized response before it occurs. For example, if a left whorl horse shows resistance to having a saddle placed on its back from the left side, switching to a right-side

presentation speeds habituation. Switching sides can also help when training a horse to lunge in a circle. For example, the first author starts a horse in a circle in the preferred direction, then switches to the nonpreferred direction. When the horse shows signs of fear (head high, head turned to the outside, etc.), he turns the back to the preferred direction. Never forcing the horse to continuously travel for long in the nonpreferred direction over time has a calming effect. Our hypothesis is that a majority of lateralized behaviors observed during riding, or during ground handling, are motivated by fear.

In summary, preventing lateral behavior before it occurs is easier than trying to correct it once it's been conditioned. Experience-dependent lateralized learning may be the way horses learn about and come to accept handling and training by humans. They first look at novelty using the eye system geared to detect possible danger. When no danger is perceived, the opposite eye system takes over and learning progresses without fear inhibiting learning. The nonintrusive neonatal foal handling method used by the first author allows foals to learn in the way they are meant to learn. Foals look at him several times from both sides before approaching and touching him for the first time. By allowing the foal to learn this way, the first author observes reduced or almost nonexistent lateralized responses to handling both at this early stage of training, and in subsequent training conducted as the horse matures.

Conclusion

The field of behavioral genetics has the potential to uncover both genetic and environmental influences on normal and abnormal behavior (Hausberger et al., 2011; Zentner and Shiner, 2012). Behavioral−genetic methods are based on a foundation of methods that are beginning to explain mechanisms of complex traits. As specific genes are identified, researchers can begin to explore how these interact with environmental factors in development. Only a few decades ago, equine scientists believed that most characteristics of horse behavior were almost entirely the result of environmental influences. Many of these characteristics are now known to be genetically influenced, in many cases to a substantial degree. Learning ability and memory, novelty seeking, activity level, fearfulness, and sociability all show some degree of genetic influence. Behavioral geneticists are interested in understanding differences among individuals. Some genetic factors may cause small differences, and others may cause large differences. Such differences may be caused by environmental factors and/or by one or many genes. Studying behavior in this way is especially important when selecting horses for breeding. Understanding how and why behaviors are developed and learned can play a large part in the breeding program. Inheritance of temperament is high, but in comparison to other domestic animals, selection for temperament in horses is less common. Breeding stallions with good performance traits

but bad temperament traits is not always a good idea. A difficult temperament can hinder performance. Balanced selection for performance traits, conformation traits, and temperament traits is important. A large body of evidence shows that selection for any single trait has unwanted effects on other traits (see Chapter 1: Behavioral genetics and animal science).

Environmental factors can affect fetal brain development; therefore, reducing stress in pregnant mares is important. Evidence showing psychological stress (fear stress) in mothers during gestation has effects on fetal brain development occurring mainly through changes in hormone profiles. Stress hormones enter the fetal bloodstream and change brain structure and function leading to increased sensitivity to stress. When fetal brain circuits are highly plastic, external influences are more pervasive.

Forced contact following birth affects emotional development, causing insecure attachment to the mare, and disrupts normal social behavioral development with peers. Forced contact can also shape how foals perceive humans. Perception of humans as "potentially threatening" may last a lifetime in reactive and fearful foals. Research shows that foals respond differently to intensive handling. Genetic differences in temperament may determine how "threatening" humans are perceived. Even short periods of postbirth handling have long-term effects. Research shows that even brief forced contact in newborn foals (assisting foals to stand and nurse), may cause foals to avoid future contact with humans. The pre- and postweaning period is a critical period of behavioral development. Conditioned fear of humans during this period by forced handling may, in some circumstances, have long-term negative effects on behavior, health, and welfare.

The ability to select a horse for a particular role, especially from a young age, is an attractive idea for breeders and trainers. Temperament tests have been created by a range of organizations and researchers to predict behavioral tendencies. The ultimate aim of temperament tests is that interested groups can perform the tests themselves and make use of the results. Unfortunately, this is not always the case. Some tests are impractical, complicated, and time consuming. Furthermore, for tests to be useful, they must be able to predict a consistency of temperament over time. As the consistency of the horse's response is what characterizes temperament, it is important that measures are chosen that clearly demonstrate this consistency. However, tests conducted over long periods of time are not practical for most trainers or people interested in purchasing a horse. In a review of the literature, the most reliable and consistent results are often found by exposing horses to sudden, unexpected novelty, or exposing them to novel objects or novel arenas that provoke sometimes severe reactions. In test situations such as these, consideration should be given to the welfare of the animals being evaluated, particularly when tests provoke fear, anxiety, or aggressive behavior. Creating a situation that causes fear in horses to learn if they have a fearful temperament, may have an effect on their future perception.

Horses do not know it is a test, all they know and learn is that humans make them afraid. The umbrella tests used by the first author to illustrate the different reactions of his horses had negative effects on one of the horses that persisted for several days following the test. For some time after the test, his calm and gentle mare, which was always easy to approach, became distrustful and reluctant to approach. Another problem with temperament tests conducted on adult horses is the influence of previous experience on temperament. Severe stress during adolescence is known to be related to temperament change, but mild stress is also common during adolescence and should not be ignored when studying temperament change. Even mild stressors can influence changes in temperament, especially when multiple events are experienced. However, evidence for stressful events during adolescence being related to changes in temperament is scarce. Adding to the scarcity of evidence of early environmental influences on temperament, stressful early experiences may be more detrimental to individuals biologically predisposed to fearfulness. All these factors are critical to understanding individual differences in temperament. Clearly, an easy and general means to assess biological factors related to temperament are needed.

A physical characteristic present at birth that remains unchanged throughout life, is easy to see, and predicts the biological foundation of temperament could have enormous implications. Hair whorls could be that characteristic. The fact that hair whorls are linked to early fetal brain development and are similar in type and distribution across at least four mammal species is remarkable. Hair whorls may provide clues to understanding pattern formation mechanisms in nervous system development. All mammal brains are basically similar in both structure and function. Even a superficial examination of the external anatomy of the brains of vertebrates reveals that most vertebrates possess the same number of brain divisions. All vertebrate brains have a brain stem, limbic system, cerebellum, and cerebral cortex. The main difference between the brains of humans and other animals is the size and complexity of the cortex. In addition, basic behaviors are similar in humans and animals, and in more complex human behaviors such as fine motor skills and language, there may be precursors in animals. Animals also respond to the environment much as humans do, reacting emotionally to others and even becoming stressed and anxious in times of danger. Therefore it requires no stretch of the imagination to assume that hair patterns and brain development follow a similar trajectory. In the past, people read too much into hair whorls in terms of horses' personalities. The way we like to explain it, hair whorls may simply predict how easily an animal gets scared. Personality, like temperament, is shaped by a complex interaction between genetic factors and experience. Hair whorls do not predict that a horse will have certain temperament or personality traits, only that the horse is predisposed to those traits, and upbringing and experience determine if those traits are expressed. In day-old foals, the first author is fond of saying, "Hair whorls are like a built-in instruction manual."

The recent discovery that the horse brain is lateralized and the two hemispheres are specialized to handle information in different ways and control different classes of behavior has the potential to solve many common problems. In our observations in cattle and horses, lateralized behaviors are signs of stress and fear. Repeatedly exposing horses to stimuli they perceive as threatening or dangerous can have profound effects on health, welfare, and productivity. Recognizing fear as the primary motivation of lateral behavior provides an alternative to correcting behaviors once established, but more importantly, a way to prevent the behaviors before they begin. Hair whorl side position and laterality have been shown in cattle and dogs, but further research is needed to find if hair whorl side position is related to laterality in horses.

Good horse trainers are talented observers of horse behavior and respond consistently to the horse's subtle cues during training. Learning the difference between individual horses and how they react differently to different situations is the key.

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Chapter 11

Genetic selection of poultry to improve welfare

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ABSTRACT
The work of Faure and Mills was groundbreaking research, which was published in the first edition of our book. This is a classic paper in behavioral genetics that everyone in the field should read, so we decided to reproduce the original chapter in the new edition. By choosing two easy-to-use behavioral tests, they created four separate genetic lines of quail. As this chapter was published in 1998, more recent research with these genetic lines has shown that the tonic immobility test does not measure all types of fear and that the social reinstatement trait is linked to other traits. At the end of this chapter, we have a list of new papers that have conducted further research on these genetic lines that show that motivational systems are complex.
In these classic experiments, quail chicks were selectively bred for the behavioral traits of fear and social reinstatement. Over a period of 20 generations, four separate genetic lines were created. They were high and low fear and high and low social reinstatement. The duration of tonic immobility was used as a measure of fear and social reinstatement was measured with a treadmill test. In this test, the time that a bird will walk on a treadmill to stay close to its flockmates is measured. Since the publication of this chapter in 1998, many scientists have carried out studies with these genetic lines. Duration of tonic immobility does not all measure all types of fear. Fear may be multidimensional because selection for tonic immobility has little effect on behavioral reactions to novelty. More recent research has also discovered linked traits such as low social reinstatement birds have stronger ultradian rhythms.

Preface to genetic selection of poultry for behavioral traits to improve welfare by Temple Grandin

This chapter was originally titled *Improving The Adaptability of Farm Animals by Selection*. I decided to add a new title that would increase the chances that this important classic paper would be found during literature

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searches. It originally appeared in the first edition in 1998, and it was reprinted in the second edition in 2014. The reason why this chapter is important was that Faure and Mills were able to use behavioral tests to select for both high and low fear birds and high and low social motivation (social reinstatement). To select for fearfulness, they used the tonic immobility (freezing) test and social motivation was measured by a clever apparatus where chicks would run on a moving treadmill to get close to their flock mates in a cage. Over 20 years ago, Faure and Mills may not have realized the importance of selecting for both a negative and a positive emotion. Today animal welfare researchers are becoming increasingly concerned that farm animals may not be experiencing sufficient positive emotions. Variations of the social reinstatement test described in 1998 are still being used today. Homberger et al. (2021) used a variation of the Faure and Mills method to measure the strength of social motivation. In their research, they assessed three personality traits of fear (tonic immobility), exploration (latency to emerge from a box test), and social motivation to rejoin flock mates. The birds they studied were gray partridges, and they discovered that the birds that were most likely to survive in the wild were high fear, high exploration, and high social motivation. The three variables measured in this test may be similar to Jaak Pansksepp's basic emotions of SEEK, FEAR, and PANIC (separation anxiety) (Panksepp, 2011).

Another variation of the Faure and Mills methods is the work by Guzman et al. (2020). In these experiments, quail were given a choice between moving toward a box containing twelve of their flockmates or moving toward three flockmates. They learned that fearfulness, as measured by a tonic immobility test, was lower in birds attracted to a larger number of companions. Both of these experiments illustrate that there is a complex interaction between fear and social motivation. The Faure and Mills work described in this chapter was one of the first studies where behavioral tests were used to select for two different emotional traits.

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Panksepp, J., 2011. The basic emotional circuits of mammalian brains: do animals have affective lives? Neurosci. Biobehav. Rev. 35, 1791−1804.

Preface by the authors from the second edition

The work of Faure and Mills was groundbreaking research, which was published in the first edition of our book. This is a classic paper in behavioral genetics that everyone in the field should read so we decided to reproduce the original chapter in the new edition. By choosing two easy-to-use behavioral tests, they created four separate genetic lines of quail. As this chapter was published in 1998, more recent research with these genetic lines has shown that the tonic immobility test does not measure all types of fear and that the social reinstatement trait is linked to other traits. At the end of this chapter, we have a list of new papers that have conducted further research on these genetic lines that show that motivational systems are complex.

Introduction

Animal welfare is a major issue in Europe. One method frequently used for solving animal welfare problems is to adapt the environment to fit the behavioral needs of the animals. However, environmental modifications are costly because economic factors are presently the predominant determinants of the design of housing systems. Another approach for improving animal welfare is to modify the animals so they are better adapted to intensive husbandry. This can be accomplished by pharmacological, surgical, or genetic methods. In this chapter, the use of genetic selection to improve adaptability to intensive rearing systems is outlined and discussed.

In Japanese quail, two types of behavior were subjected to genetic selection: social reinstatement (the tendency to rejoin flockmates) and fearfulness. These two traits interact and determine how a bird may behave in different situations. Information on the inheritance of behavioral traits in quail may provide insights into the inheritance of these traits in other species. Before we discuss genetic selection, we will briefly summarize nongenetic approaches to improving welfare.

Pharmacology

This approach was first tested in the 1950s and 1960s after neuroleptic drugs were discovered (Gilbreath et al., 1959). Pharmacology was used in an attempt to calm animals during harvesting and transport. The early use of neuroleptic drugs as tranquilizers was unsuccessful. Furthermore, consumer awareness of drug residues makes such methods unacceptable for commercial use. However, pharmacological research can provide useful insights into animal behavior, adaptability, and brain function.

Surgery

Surgical methods such as dehorning, beak trimming, or tail docking in domestic animals can prevent welfare problems such as pecking injuries and

tail biting. However, these methods are criticized by welfarists because pain is experienced at the time of the operation. Gentle et al. (1991) found that beak trimming in poultry may cause pain for a prolonged period of time after the operation.

Ontogeny

Some problems that make it difficult for production animals to adapt to their rearing environment can be improved to some degree by ontogenetic processes such as handling or environmental enrichment (Jones, 1982; Jones and Faure, 1981; Jones et al., 1991). Ontogenetic manipulation involves the manipulation of the animal's early experiences. The disadvantage of these procedures is that they are environmental rather than genetic and they must be repeated in subsequent generations.

Genetic selection

Adaptation of animals to their environment can also be improved by genetic selection. This method has the advantage of being applied to relatively small numbers of carefully selected breeding animals with subsequent genetic gain being transmitted to their descendants. This chapter will focus on the use of genetic selection and its applicability to practical problems that occur in intensive husbandry systems. Genetic selection can be used to improve welfare. Although it is unlikely that genetic selection alone can be used to solve all welfare problems, a combination of improvement of the environment and ontogenetic processes, together with the use, at least in emergency cases, of pharmacology or surgery is probably the only realistic way of improving domestic animal welfare (see Chapter 9).

Genetic selection has influenced the processes of domestication in at least two ways (see also Chapter 2). These are natural selection and artificial selection for specific traits. When animals were first domesticated, natural selection was probably the main cause of genetic change. This means that animals showing low levels of adaptation to the new, man-made conditions had poorer fitness and their genes were eliminated from the gene pool of the next generation (Faure et al., 1992). This type of selection is likely to be efficient only when a high proportion of animals are carrying genes of low value in the new artificial environment. For example, animals with high fear and a flighty, nervous temperament may be so stressed that they fail to breed in captivity. Subsequently, the failure of these animals to breed in captivity probably resulted in natural selection for lower levels of fearfulness. During the early stages of domestication, farmers were able to recognize individual traits in their animals and prevented most animals exhibiting undesirable characteristics from reproducing. For example, a very aggressive bull that endangered the farmer would probably be culled. When flocks or herds

become too large to allow recognition of individual animals, this method was no longer applicable. For both practical and economic reasons, animal breeding was subsequently concentrated on specific traits, irrespective of their relationships to other traits or concerns for animal welfare.

A more sensible solution would be to select for general adaptability and characteristics that are expressed in a wide variety of environments. High appetite is an example of a trait that can be expressed in a wide range of environments; environmental change cannot mask the effects of genetic selection. On the other hand, perching is a behavior that can only be expressed in certain environments. Obviously, perching behavior cannot be expressed if perches are not available. Faure and Jones (1982a, b) found that the perching behavior of chickens differs between strains. This suggests that it should be possible to select lines with high and low levels of perching behavior. Although selection for low levels of perching behavior might improve the adaptation of domestic hens to battery cages, if floor pens or aviaries are used it might be advantageous to promote high levels of perching behavior (Appleby et al., 1988). Either type of selection would lead to very specialized lines of chickens that would be undesirable given the diversity of modern husbandry systems.

We chose to select for two characteristics that are indicators of general adaptability and are behaviorally expressed in most environments: fear and social reinstatement behavior (see Chapter 2 for further information on fear). Social reinstatement is the tendency of animals to flock and stay close to conspecifics (flockmates). Fear and social motivation are basic motivational systems in a wide variety of species. We chose to use the Japanese quail as a model of other species.

Selection procedure

The measurement of behavioral traits is often very time consuming, and selection requires behavioral measures of large numbers of animals (Faure, 1981). Therefore we chose to use relatively short tests (no longer than 5 minutes). Social reinstatement behavior was measured using the treadmill test (Mills and Faure, 1990). The apparatus has two compartments, one containing flockmates and the other a treadmill on which the test animal is placed (Fig. 11.1). If the test animal moves toward the other birds, the treadmill moves in the opposite direction. The movement of the treadmill serves as a measurement of the effort made by the test animal to reinstate social contact. If the test animal moves away from the other birds, the time spent in the "far end" of the apparatus is also measured. Tests were performed when chicks were between 6 and 8 days old and lasted 5 minutes.

High and low levels of fear were determined using the tonic immobility reaction (Jones, 1987). In this test, the chick is placed on its back in a U-shaped cradle covered with several layers of white cloth and is restrained by

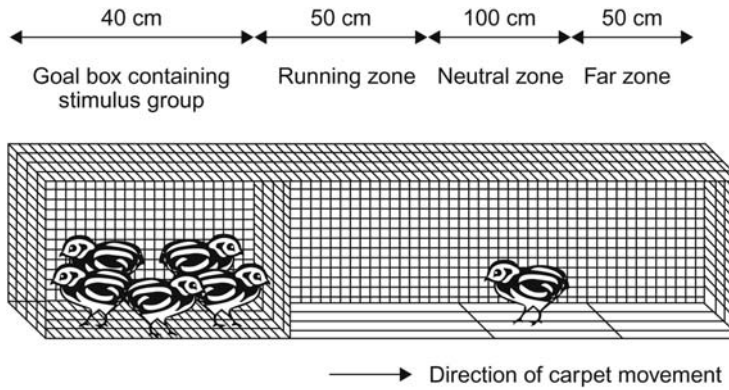


FIGURE 11.1 Schematic plan of the treadmill apparatus used to measure social reinstatement behavior in Japanese quail chicks.

the experimenter for 10 seconds. If the chick remains immobile for 10 seconds after the removal of restraint, tonic immobility is considered to have been induced. Animals that remain immobile for "long periods" are considered to be fearful (Jones and Mills, 1982; Mills and Faure, 1986). If the chick rights itself within 10 seconds, it is considered that TI (tonic immobility) has not been induced and a further attempt at induction is made. If five attempts at induction are unsuccessful, the duration of TI is considered to be 0 and the number of attempts at induction is recorded as five. The TI test is performed when the chicks are 9−10 days old. For the purpose of selection, the maximum duration of TI was set at 5 minutes.

Selection over 20 generations resulted in lines showing either high or low levels of social reinstatement (SR) behavior and long or short duration of TI. Appropriate control lines (CSR and CTI) were also maintained (Mills and Faure, 1991). At each generation, 20 males and 40 females were kept in cages containing one male and two females. These animals were the breeding population of each line. Approximately 200 quail chicks were hatched at each generation. Selected animals from each hatch served as the breeding population for the next generation. The sire of every chick was identified (one male per cage) and within dam families were identified on the basis of egg color, shape, and size. Morphological characteristics of eggs are very constant within females but very different between females. Furthermore, each chick was individually banded at hatching. Wing bands were used to identify the animals throughout their lives. In the SR lines, the measure of SR behavior (measured in arbitrary units) is the difference between the distance run on the treadmill minus the "far" time. It was shown in a preliminary experiment that the distance run and the far time were equal when the animals were tested in the treadmill apparatus without stimulus animals, resulting in a score of 0.

To ensure that selection for TI did not influence SR behavior or vice versa, all animals were tested for both TI and SR behavior and the scores for each trait were subjected to regression analysis. In the lines selected for high levels of social reinstatement behavior, the SR score was calculated on the regression graph with the duration of TI. The selection criterion was the deviation of each individual data point from the regression line. In the tonic immobility lines (high fear), the measure of TI was the time taken by the birds to right themselves. This value was regressed on the chick's score for social reinstatement behavior. Again, the selection criterion was the deviation of each data point from the regression line. This transformation reduced the risk of coselection for the two traits due to the correlation between social reinstatement and tonic immobility. Breeding values were calculated taking into account not only the individual values but also those of full siblings (Mills and Faure, 1991). The two control lines were bred from one son chosen at random from each father and one daughter from each mother. Lines were selected as above until the 16th generation. However, from generation 17 onward, "skip a generation" selection was employed. In the nonselected generation, breeding animals were chosen at random and used for the control lines.

Selection results

Selective breeding for high and low social reinstatement and high and low tonic immobility produced significant changes in behavior. In the first generation, the mean (± SD) value for distance run on the treadmill was 14.4 m (± 8.9). After 20 generations of divergent selection for high or low levels of social reinstatement behavior, values were 59.0 (± 27.3) m run in the high SR line, 7.4 (± 11.4) meters run in the control SR line, and 4.0 (± 8.9) m run in the low SR line (Fig. 11.2). The distribution of

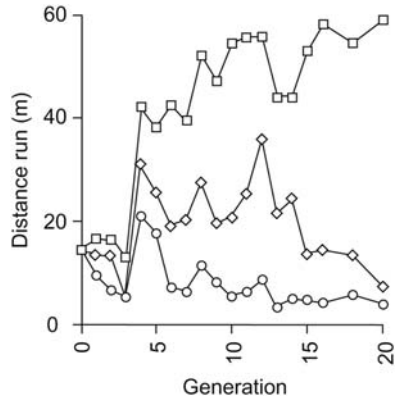


FIGURE 11.2 Distance run on the treadmill in lines of Japanese quail selected for high or low levels of social reinstatement behavior over 20 generations (□) HSR, (◇) CSR, (○) LSR.

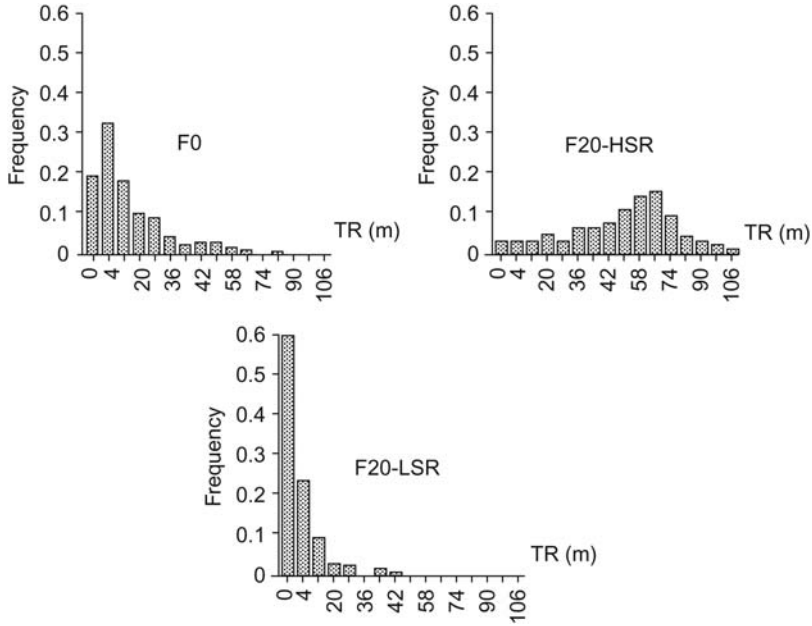


FIGURE 11.3 Distribution of distance run on the treadmill in lines of Japanese quail selected for high or low level of social reinstatement behavior. The graph shows the distributions for the F0 and F20 generations.

values for the distance run was completely different between the 1st and 20th generation of the high SR and low SR lines (Fig. 11.3). From the 10th generation of selection onward, the standard deviations of the high SR and low SR lines ceased to overlap. This would seem to indicate that the high social reinstatement (HSR) and the low social reinstatement (LSR) birds had become distinct genetic lines.

In the SR lines, the time spent in the far zone of the runway was identical up to the eighth generation of selection. Thereafter, the lines started to diverge with low SR birds showing the longest times and high SR birds the shortest times. Control SR birds had intermediate times (Fig. 11.4). Over 20 generations of selection, the duration of the tonic immobility response was remarkably constant and similar in all three of the lines selected for social reinstatement lines (Fig. 11.5). This shows that tonic immobility and social reinstatement are separate traits that are genetically independent of each other.

In the first generation (F0) of the lines to be selected for the differing duration of TI responses, the mean ($\pm$ SD) value for the duration of TI was 50.5 ($\pm$ 52.9) seconds. After 20 generations of divergent selection, TI scores

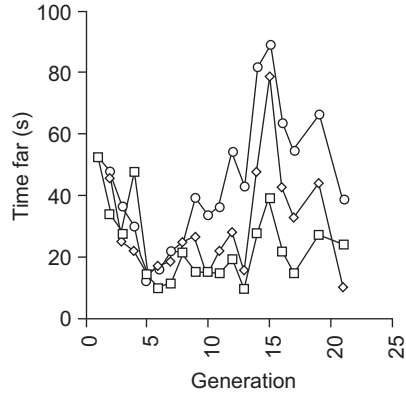


FIGURE 11.4 Time far in the treadmill lines of Japanese quail selected for high or low level of social reinstatement behavior over 20 generations. (□) HSR, (◇) CSR, (○) LSR.

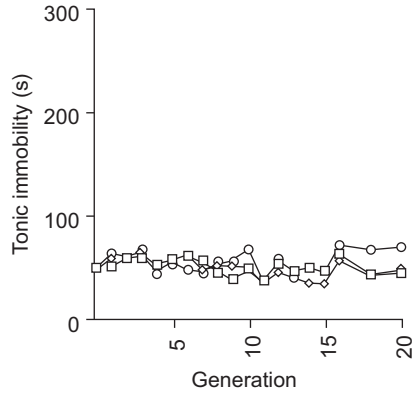


FIGURE 11.5 Duration of tonic immobility in lines of Japanese quail selected for high or low level of social reinstatement behavior over 20 generations. (□) HSR, (◇) CSR, (○) LSR.

were 215.6 (± 91.9) seconds in the long TI line, 50.8 (± 38.4) seconds in the control TI line, and 9.3 (± 11.6) seconds in the short TI line (Fig. 11.6).

The tonic immobility response in the long TI (high fear) line was approximately 3.5 minutes compared to only 9 seconds in the short TI (low fear) birds. The distribution of scores for the duration of TI in the first generation was completely different from those in the 20th generation of the long TI and short TI lines (Fig. 11.7). Starting from the eighth generation onward, the standard deviations of the long TI and short TI lines ceased to overlap. This implies that the long tonic immobility (LTI) and the short tonic immobility (STI) lines had also become distinct genetic lines.

After 20 generations of selection, the number of inductions necessary to obtain tonic immobility increased in the short TI line (3.88 ± 1.47), remained

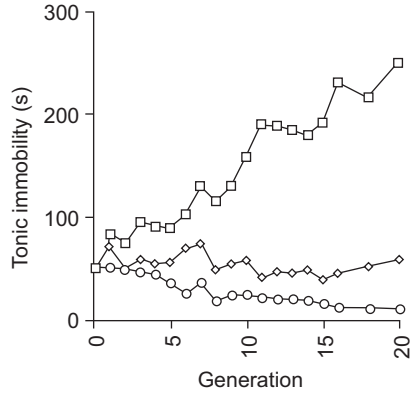


FIGURE 11.6 Duration of tonic immobility in lines of Japanese quail selected for long or short duration of tonic immobility over 20 generations. (□) LTI, (◇) CTI, (○) STI.

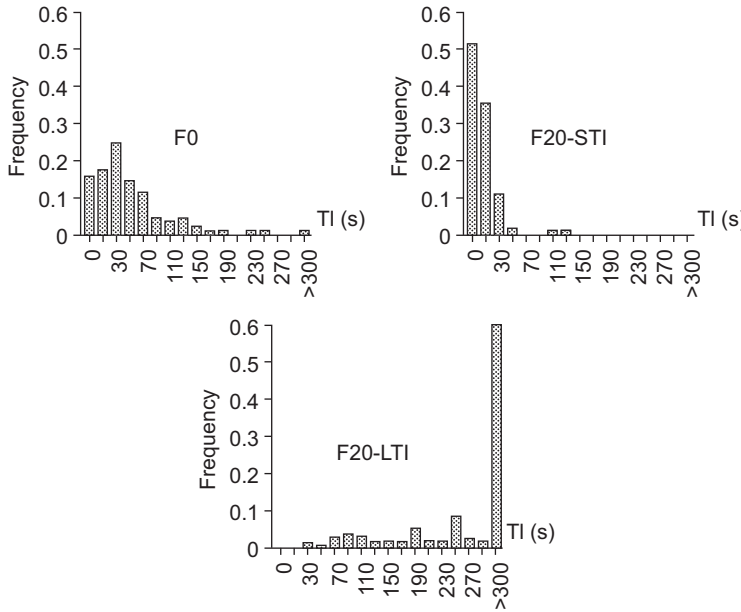


FIGURE 11.7 Distributions of the duration of tonic immobility in lines of Japanese quail selected for long or short duration of tonic immobility. The graph shows the distributions for the F0 and F20 generations.

relatively constant in the control TI line (1.47 ± 0.75), and decreased in the long TI line (1.04 ± 0.20) (Fig. 11.8).

The distance run to reinstate social contact by the different TI lines remained relatively constant and similar in all three lines up to the eighth

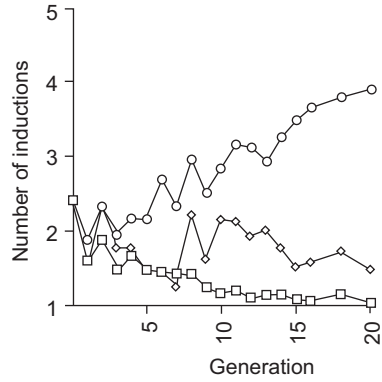


FIGURE 11.8 Number of inductions required to induce tonic immobility in lines of Japanese quail selected for long or short duration of tonic immobility over 20 generations. (□) LTI, (◇) CTI, (○) STI.

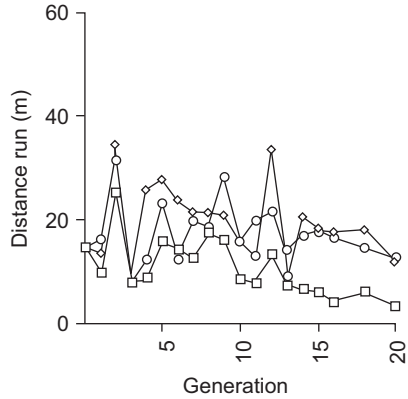


FIGURE 11.9 Distance run on the treadmill in lines of Japanese quail selected for long or short duration of tonic immobility over 20 generations. (□) LTI, (◇) CTI, (○) STI.

generation. Thereafter, distance run started to decrease in the long TI line (Fig. 11.9). This finding is not surprising as Jones et al. (1991) showed that long tonic immobility chicks (high fear) have reduced activity levels when placed in a novel environment. Reduced activity is due to increased fear levels and leads to increased freezing and the suppression of general activity.

In both the SR and TI lines, responses to selection were asymmetrical (Figs. 11.2 and 11.6). These asymmetrical responses are likely to have been due to an artificial ceiling or actual floor limits to selection. For example, the duration of TI cannot be less than 0 (a reduction of 100% relative to the first generation (F0)), whereas the upper limit of TI was 300 seconds (an increase of approximately 600% relative to the F0 score).

Stability of the selection results

One problem with selection for a specific trait at a certain age is that the response to selection may be specific to the age at which the measure was made. We, therefore, verified that behavioral differences observed at 6−10 days old were also present in older animals. Quail were tested for tonic immobility and distance run on the treadmill using the same methods employed for the selection of breeding animals. However, the maximum duration of TI measurements was lengthened to 30 minutes. Different animals were tested at 1, 2, 4, 6, or 10 weeks old (Launay et al., 1993; Launay, 1993).

The duration of tonic mobility varied with age (Fig. 11.10). However, the scores of the long TI and short TI lines differed significantly at all ages.

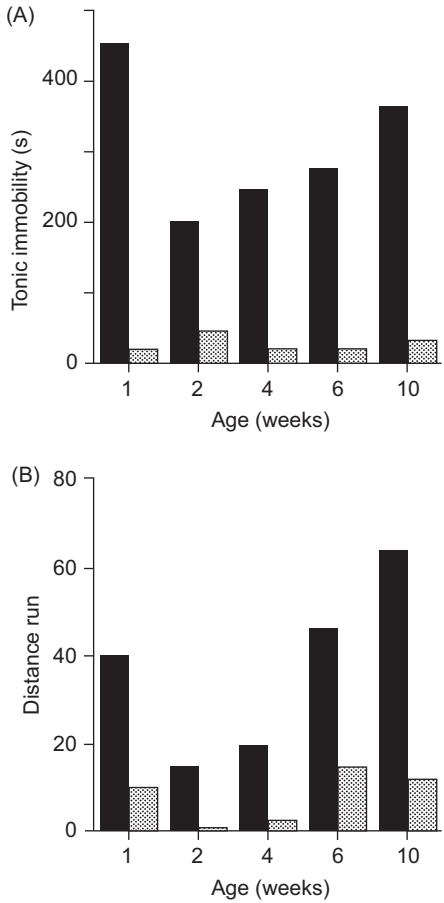


FIGURE 11.10 Duration of tonic immobility in 1-, 2-, 4-, 6-, or 10-week-old quail chicks selected for long (■) or short (▨) duration of tonic immobility over 13 generations (A) and distance run on the treadmill by 1-, 2-, 4-, 6-, or 10-week-old quail chicks selected for high (■) or low (▨) levels of social reinstatement behavior over 13 generations (B).

Comparison of Fig. 11.10A and 11.6 shows that the maximum duration of tonic immobility in the long TI line was underestimated because TI testing was terminated after only 5 minutes. Data shown in Fig. 11.10 are for quail from the 13th generation of selection; the mean duration of tonic immobility in the long TI line at 1 week old was over 400 seconds, whereas the truncated values obtained in the selection program were less than 200 seconds.

Distance run on the treadmill was also age dependent (Fig. 11.10B). However, the differences between the high SR and low SR lines were significant at all ages tested. As differences in the characteristics selected persisted into adulthood, it seems likely that genetic selection has modified the behavior of the birds throughout their lives rather than only at a specific age.

Fear reactions

Ratner (1967) proposed that fear (antipredator) reactions may be dependent on the proximity to the predator. When the predator is distant, the animal's first reaction is freezing. Freezing behavior may serve to reduce the likelihood of detection. When the predator approaches, flight is the prevalent reaction. If the bird is not able to escape from the predator, it shows fight responses. If this fails, tonic immobility follows. The open-field test provides a means for treating this hypothesis.

The open-field test is commonly used as a means of measuring fear in birds and mammals (Hall, 1934). The open-field test involves the introduction of an animal into a novel environment. Being placed in an open field induces a low level of fear due primarily to the novelty of the test area. Behavior in the open field may also reflect social reinstatement tendencies because an animal that is isolated in the arena may suffer a motivational conflict between fear and the desire to reinstate social contact with its companions (Faure et al., 1983; Gallup and Suarez, 1980). Therefore it was important to test the effects of selection for TI and SR behavior on the behavior in the open field. This was performed using a long-duration open-field test (30 minutes split into three periods of 10 minutes; Launay, 1993). The various parameters used to measure open-field behavior were derived from computer-based trajectometric analysis of video images (see Mills et al., 1990), and direction observation of behavior (Launay, 1993). During the first 10-minute period of the open-field test, the behavior of short TI (low fear) and high SR lines was of an active type (i.e., short latencies, high activity), whereas the behavior of chicks of the long TI (high fear) and low SR lines was the inverse (Fig. 11.11). Immediately after being placed in the open field, the short TI (low fear) and the high SR line birds walked or ran around the arena whereas the long TI (high fear) and the low SR birds did just the opposite. These birds took a long time to start moving around the open field, and they moved very little during the first 10 minutes of the rest.

During the second 10-minute period of the test, discriminant analysis (a powerful statistical test) permitted discrimination between lines. At least for

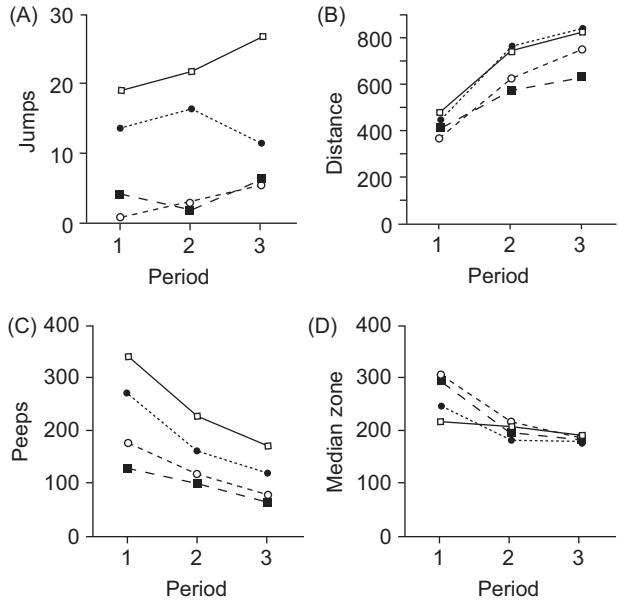


FIGURE 11.11 Changes in four behavior patterns in three successive 10-minute periods in open-field test. (A) Number of jumps, (B) distance run, (C) number of peeps, (D) time in the median zone. (□) HSR, (■) LSR, (○) LTI, (●) STI.

certain behavior patterns, long TI and low SR line chicks began to resemble short TI and high SR line chicks (Fig. 11.12). However, during the third 10-minute period of the test, high SR and low SR lines could still be discriminated, but the long TI and short TI lines could not. These results indicate that even though the behavior of short TI and high SR (or of long TI and low SR) were similar during the first period of the test, the motivational states of the lines were different. These differences in motivation can be explained by the fact that the behavior of the long TI and short TI lines are primarily motivated by differing levels of fear, but the differences between the two lines wane after habituation to a novel environment. This illustrates why it was important to conduct the open-field test with three different time periods. Fear behavior, which is observed after a short period in the open-field test, disappears after the birds habituate. Conducting the tests in three time periods also provides information on the time required for habituation in the different lines.

The differences between chicks of the high SR and low SR lines are primarily due to differences in social reinstatement tendencies that do not attenuate or change during the 30-minute test period. As suggested by other authors (Faure et al., 1983; Gallup and Suarez, 1980), these results demonstrate that open-field behavior is the expression of two different motivations: fear that leads to inhibition of activity, and social reinstatement that induces

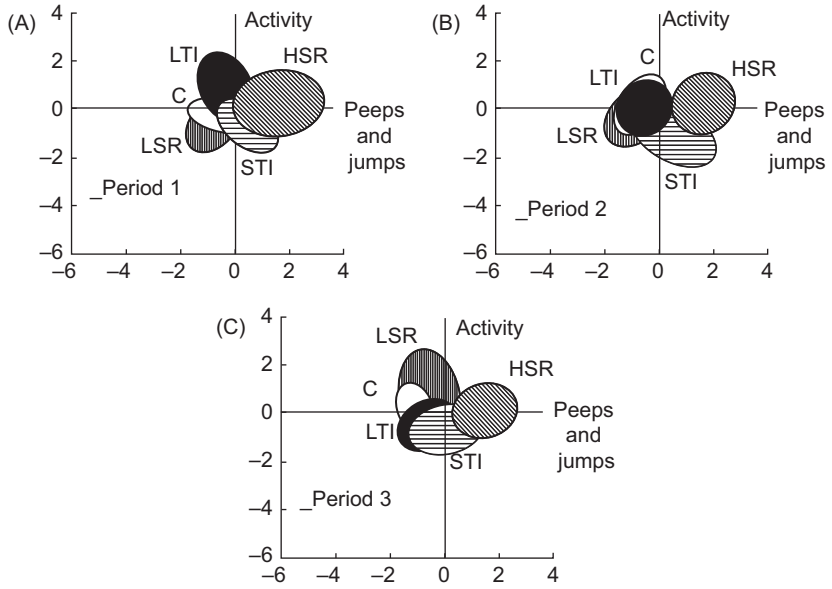


FIGURE 11.12 Discriminant analysis of the open-field behavior of lines of Japanse quail selected for long or short duration of tonic immobility and for high or low levels of social reinstatement behavior over 12 generations.

high levels of activity related to the search for social companions (see also Mills et al., 1993). This also demonstrates that short open-field tests may not allow differentiation between fear and social reinstatement tendencies.

Handling and environmental enrichment are known to reduce fear levels in chickens (Candland et al., 1963; Jones, 1982; Jones and Faure, 1981). When these treatments were applied to chicks of the long TI and short TI lines of quail (Jones et al., 1991), they were effective in reducing fear levels in both the lines (Fig. 11.13). However, there was a tendency for handling and environmental enrichment to be more effective in the short TI lines than in the long TI line. This effect might be explained by a reduction in information input in the long TI quail during handling or enrichment, attributable to greater fear-induced behavioral inhibition and fear.

The long TI and the short TI lines also had very different reactions to capture by a person. Two hundred and fifty quail of the long TI and short TI lines were reared together in a floor pen and were caught at three successive ages (2, 4, and 6 weeks old). Birds were captured at random, and the short TI (low fear) animals were easier to catch and were captured first at all the ages tested (Fig. 11.14).

Ratner (1967) proposed that the fear (antipredator) reaction is dependent on predator proximity. At long distances, the first reaction is freezing, which may reduce detectability. At closer distances, flight reaction predominates. If

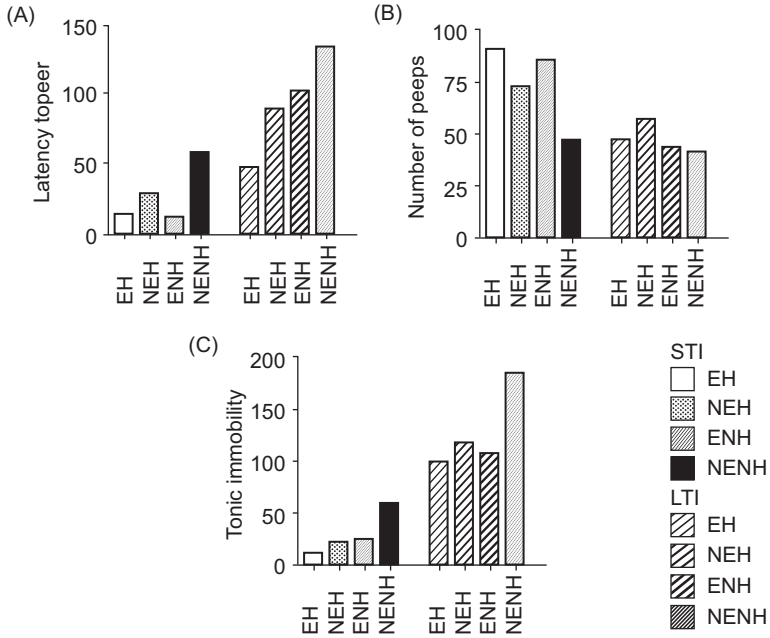


FIGURE 11.13 Variation in measures of fear in response to handling and environmental enrichment in lines of Japanese quail selected for long or short duration of tonic immobility over nine generations (EH: enriched, handled; NEH: nonenriched, handled; ENH: enriched, nonhandled; NENH: nonenriched, nonhandled). (A) Latency to peep, (B) number of peeps, (C) tonic immobility.

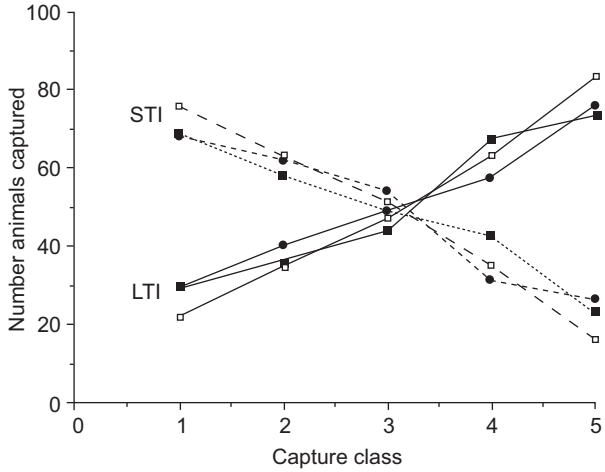


FIGURE 11.14 Proportion of birds of each line in five 100-animal capture classes of quails selected for long or short duration of tonic immobility over 16 generations at three ages: 1, two weeks old; 2, four weeks old; 3, six weeks old. LTI: (■) Age 1, (●) Age 2, (□) Age 3; STI: (●) Age 1, (■) Age 2, (□) Age.

the flight is unsuccessful, fighting and tonic immobility may follow. The least-studied behavior in this chain of reactions is the fight reaction. To study this reaction, we placed quail in a crush (squeeze) cage similar to that described by Satterlee and Johnson (1988). In this apparatus, the animal's movement is restricted but bouts of fight reactions occur when the bird struggles to escape from the rush. The average number of struggles was 6 in the long TI line and 56 in the short TI line. Hence, the reactions described by Ratner (1967) as antipredator responses were more difficult to induce in the short TI line. Differences in the threshold for the release of fear-related behavior may explain these line differences.

In the open field, where environmental novelty induces low-to-moderate levels of fear (see also Turro-Vincent et al., 1995, for a discussion of novelty and fear responses), short TI line chicks initially showed less freezing but the difference between the short TI and long TI lines disappears toward the end of the 30-minute test when the habituation took place. In a capture experiment where a man had the quasi-role of predator short TI line birds showed fewer flight reactions and were captured first (Mills and Faure, 1995). In situations that stimulate capture (crush cage and inductions of TI), short TI line birds show strong flight reactions and do not readily show the TI reaction. Furthermore, if the short TI line birds do show tonic immobility, their duration is short.

Social behavior

The influence of selection for social reinstatement behavior on the tendency to approach flockmates has been measured in various ways. Launay et al. (1991) compared approach tendencies toward paired goal boxes that either were empty or contained a group of conspecific (flockmate) chicks. High SR line chicks spent more time close to goal boxes containing conspecifics than low SR line chicks (Fig. 11.15). Mills et al. (1993) and Francois and Mills (1996) compared interindividual distances in mixed or same-line pairs of high SR and low SR line chicks in open-field tests. At 1 and 3 weeks old, interindividual distances were greatest in the low SR/low SR pairs, intermediate in high SR/low SR line pairs, and lowest in the high SR/high SR pairs, intermediate in high SR/low SR line pairs, and lowest in the high SR/high SR line pairs (Fig. 11.16). In other words, low social reinstatement chicks stand away from each other and high social reinstatement chicks group together. At 6 weeks old, there were no significant differences in interindividual distances between fixed or same line pairs. Given that genetic line differences in treadmill behavior persist into adulthood, this last finding may appear surprising. However, age-related variations in interindividual distances occurred only in the high SR lines. Low SR line birds appeared to space themselves at random. This implies that social factors influenced spacing in high SR line birds but not in low SR line birds.

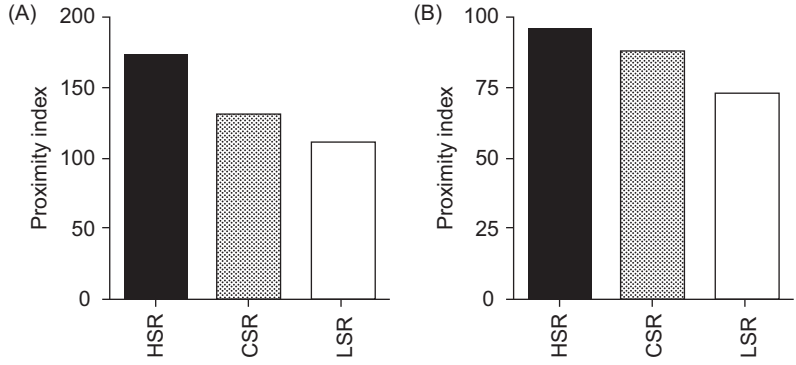


FIGURE 11.15 Proximity indices in lines of Japanese quail selected for high or low level of social reinstatement behavior over five generations. (■) HSR, (▨) CSR, (□) LSR.

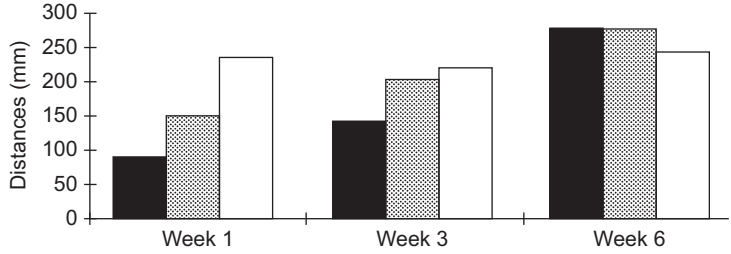


FIGURE 11.16 Interindividual distances in the open field between pairs of 1-, 3-, and 6-week-old quail from lines selected for high or low level of social reinstatement behavior over 18 generations. (■) HSR/HSR, (▨) HSR/LSR, (□) LSR/LSR.

In the high and low social reinstatement lines, an age-related variation in behavior was found using the time budget test. In this test, quail of the high SR and low SR lines were given a long-term choice between being in social contact with conspecifics (flockmates) without access to food and water and having access to food and water in the absence of conspecifics. At 1 week old, chicks of both lines spent most of their time (about 80%) in proximity to conspecifics; the time spent close to flockmates decreased with age in both lines (Fig. 11.17). However, this effect was more pronounced in the low SR line than in the high SR line. At 4 and 6 weeks old, high SR line chicks spent more time near conspecifics than did low SR line chicks.

Under the three experimental conditions employed, the results show that the social motivation of the high SR animals varied with age and test conditions. In the treadmill test, line differences were stable from hatching until 10 weeks old. Line differences were also present in the young animals but they disappeared by 6 weeks old. However, in the time budget rest, line differences were absent in the young birds but appeared at 4 and 6 weeks old.

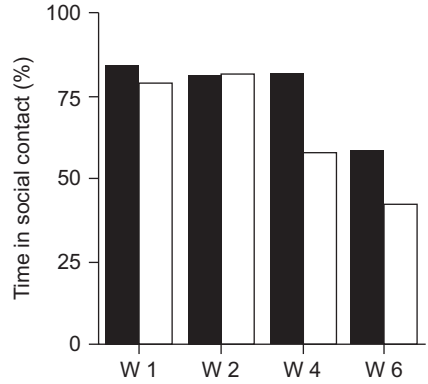


FIGURE 11.17 Time spent in social contact by 1-, 2-, 4- or 6-week-old quail from lines selected for high or low level of social reinstatement behavior over 18 generations. (■) HSR, (□) LSR.

The results of the time budget experiment can probably be explained by the fact that the birds being tested were familiar with each other and the test environment. This was not the case in the treadmill and open-field tests.

The treadmill and time budget tests did not permit the expression of aggression because the two birds were separated from one another by a wire mesh partition. Under these conditions, these differences between adult high SR and low SR quail. In the open-field test, aggression can be expressed because there is no barrier between birds, and adult interindividual distances are identical in high SR and low SR lines. To test for differences in aggressiveness, paired encounters were staged between low SR and high SR pairs of adult birds. High SR line birds showed more aggression (particularly attack) than the low SR line birds (Magnolon, 1994; Francis, unpublished data).

Another situation in which social motivation can be measured is tests of social facilitation. In many species, social facilitation influences feeding behavior. In quail, social facilitation occurs when one bird sees another bird doing something and then imitates or increases its own expression of that behavior. To test social motivation in each of the four selected lines, groups of three quail were presented with a novel (colored) food, either within sight of birds called teachers or in their absence. Teacher birds were preadapted to the colored food and readily consumed it. The birds being tested were also familiar with the teacher birds and control tests were run using the normal food of the test birds. There were no differences in the four lines in the control situation (normal food in the presence or absence of teachers), in time elapsed to start eating, nor in the time spent at the feeder. In the novel food without teachers situation, significant differences were found between the long TI and short TI lines (Fig. 11.18). Long TI (high fear) birds took longer to eat. The longer latency to begin eating the colored food in the high tonic

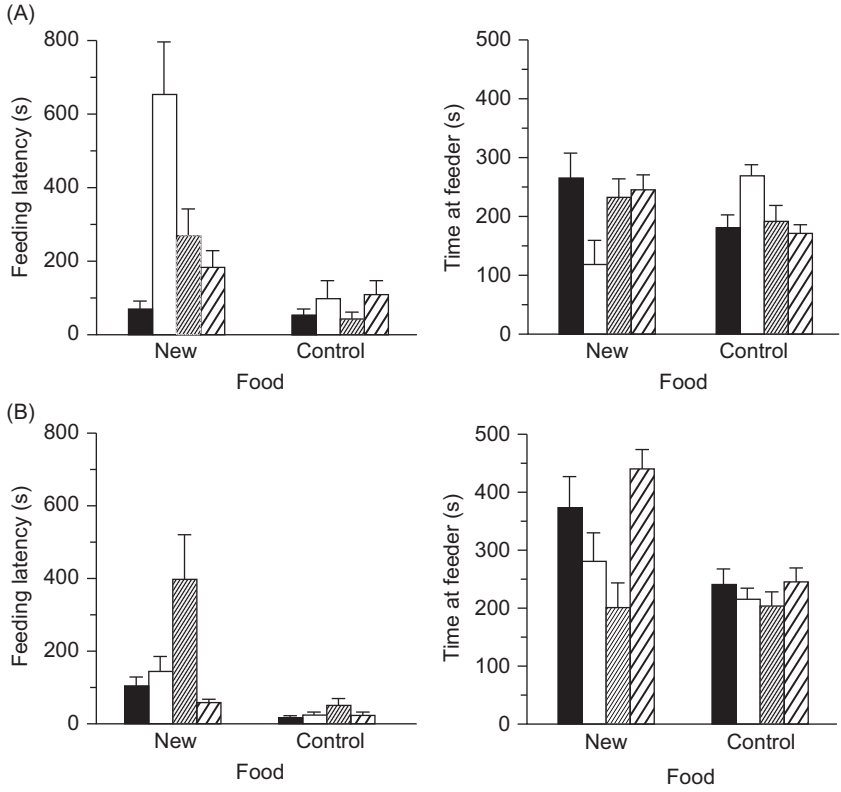


FIGURE 11.18 Feeding latency and time at the feeder during the first contact with familiar or novel food in the presence or absence of animals acquainted with the food tested in lines of quails selected for long or short duration of tonic immobility and for high or low levels of social reinstatement behavior over 14 generations. (■) STI, (□) LTI, (▨) LSR, (▨) HSR. (A) Without teachers, (B) with teachers.

immobility chicks is possibly explained by higher neophobia (fear of novelty) in this line. In the novel food with teachers present test, the high SR and low SR lines differed. The low social reinstatement line had longer (latency) times to start eating and spent less time at the feeder. In this social situation, high SR line chicks appeared to learn from the teachers whereas the low SR line chicks did not.

Stress reactions

Stress reactions can also be induced by frightening stimuli (Toates, 1995) or by modifications of the social environment (Gross and Siegel, 1985; Jones and Harvey, 1987). In the four genetically selected lines of quail, the birds selected for high and low levels of fear and the birds selected for high and

low levels of social reinstatement tendencies should, theoretically, react in different ways to different types of stress-inducing stimuli. If this model has validity, two predictions can be made: (1) the long tonic immobility (high fear) line is likely to be the most reactive to novelty or frightening manipulation of the test environment, and (2) the high SR lines will be more reactive to manipulations of the social environment.

To test these hypotheses, birds from the four lines were subjected to three potentially stressful situations and plasma corticosterone (stress hormone) levels of each animal were measured. The tests were as follows: (1) the introduction of a novel stimulus (an inflated balloon into the home cage), (2) restraint in a crush cage, and (3) isolation in a novel environment (Fig. 11.20). The introduction of the balloon into the home cage (Fig. 11.19) induced a significant increase in plasma corticosterone levels only in the long TI line (Launay, 1993). Presumably, the more stressful experience of restraint in a crush cage as described by Satterlee and Johnson (1988) induced different behavioral and physiological reactions in the long TI and short TI lines (Jones et al., 1994). During restraint in the crush cage for a period of 15 minutes, the short TI (low fear) birds showed more active struggling reactions (55.7) compared to the long TI (high fear) line birds (6.4) that typically show tonic immobility or freezing reactions. In the two selected lines and the control line, restraint induced an increase in plasma corticosterone levels that were greater in the short TI line compared to the control and long TI lines (Fig. 11.21) (Jones et al., 1994; Remingnon et al., 1996). The higher level of struggling in the short TI (low fear) line was predictable. However, the relatively low corticosterone response in the long TI (high fear) line was unexpected because tonic immobility and freezing were

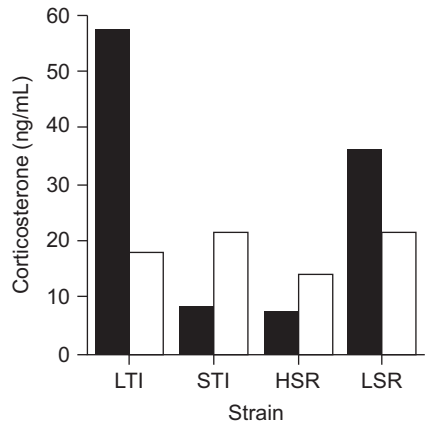


FIGURE 11.19 Plasma corticosterone levels 15 min after the introduction of a balloon into the cage of quails from lines selected for long or short duration of tonic immobility and for high or low levels of social reinstatement behavior over 14 generations. (■) Stressed, (□) control.

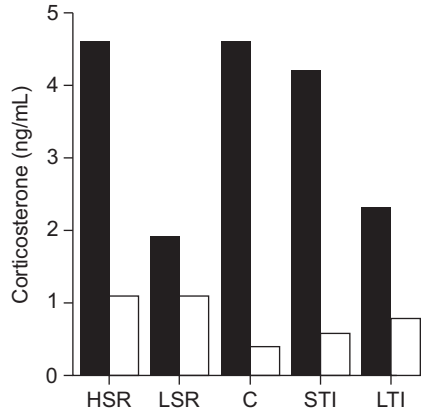


FIGURE 11.20 Plasma corticosterone levels after 15 min of social isolation in lines of quails selected for long or short duration of tonic immobility and for high or low levels of social reinstatement behavior over 14 generations. (■) Stressed, (□) control.

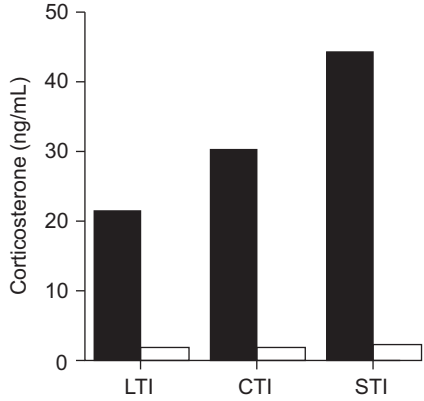


FIGURE 11.21 Plasma corticosterone levels after 15 min in a crush cage, in lines of quails selected for long or short duration of tonic immobility and for high or low level of social reinstatement behavior over 18 generations. (■) Stressed, (□) control.

likely to be induced by the highly stressful nature of the crush cage test. The only explanation we can suggest for this is that the TI reaction not only protects animal from predators but also allows the animal to cut itself off from noxious or frightening stimuli. It appears that the struggling and fighting displayed by the low TI birds were a more stressful response (as measured by stress more levels in the blood), compared to the freezing response expressed in high TI birds. Each has adaptive significance. Freezing behavior protects birds from being detected by the predator, and fighting and struggling may also protect the bird once the predator has caught it.

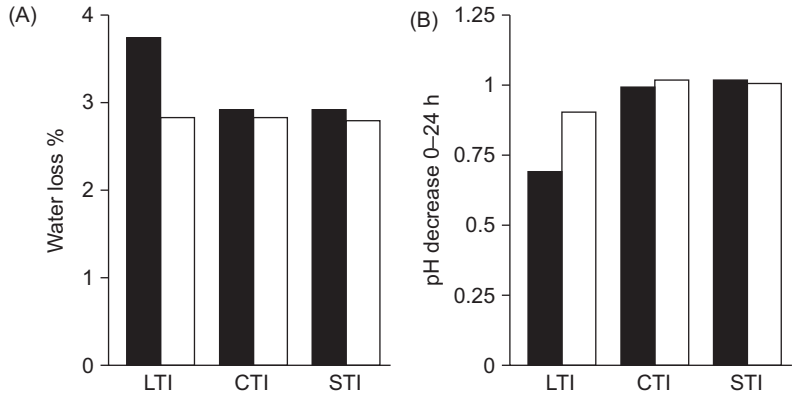


FIGURE 11.22 Meat water loss (A) and 24-h *post mortem* pH decrease (B) following 15 min in a crush cage in lines of quail selected for long or short duration of tonic immobility and for high or low levels of social reinstatement behavior over 18 generations. (■) Stressed, (□) control.

Stress reduces meat quality by increasing water loss and decreasing pH (Remingnon et al., 1996). When water loss and pH decrease 24 hours *post mortem* were measured in the muscle from three lines (short TI, control, and long TI), control values for these two measures were identical. However, following stress muscle from long TI birds showed a higher percentage of water loss and a lower pH decrease than did tissue in their unstressed counterparts, from stressed or unstressed controls, and from the short TI line (Fig. 11.22). In quail raised for meat, long TI birds may have reduced meat quality because they may be more likely to be stressed by handling and transport before slaughter.

Neurobiology

Little is known about the effects of genetic selection on brain function. Selection for behavioral characteristics should theoretically result in neurophysiological traits. We have studied the number and binding activity of neurotransmitter receptors in the forebrains of long TI (high fear) and short TI (low fear) birds in an attempt to determine neuroanatomical and biochemical differences between lines.

Neurotransmitters are "messenger" substances secreted by nerve endings that cross the synapses (the junctions between nerve endings) and bind to their specific receptor sites on adjoining nerve endings. For example, serotonin transmitters bind to serotonin receptors like a key fitting in a lock. The brain contains many different neurotransmitters and corresponding receptor sites. These transmitters have a wide variety of influences on behavior ranging from calming to excitatory effects. Behavioral effects can be affected by both the number of receptor sites for a certain transmitter and the affinity of the receptor sites. To explain it very simply, a greater number of receptors or

an increase in the affinity of binding at each site will influence the "strength" of transmission. Documentation of a relationship between genetic selection for traits, such as high or low levels of fear and the number and affinity of different receptor sites, indicates that genetic selection affects brain mechanisms that influence behavior.

One of the brain areas involved in fear reactions in birds is the archistriatum (Davies et al., 1997); the effects of lesions of this area have been studied (Davies et al., 1997; Grignard, 1996). In young animals, archistriatal lesions have limited effects in the short TI, high SR, and low SR lines. However, such lesions decrease fear reactions in the highly fearful long TI chicks as measured by decreased latency to peep, increased number of peeps in the hole in the wall box test, and a decreased duration of TI (Davies et al., 1997). Lesioned subadult long TI line quail were easier to capture and had reduced avoidance of novel objects, people, and unfamiliar food (Grignard, 1996). The archistriatum has undergone some form of change during selection, and further research will attempt to determine the nature of these changes.

Hoggs et al. (1994, 1996) showed that the forebrains of short TI line (low fear) birds contain more benzodiazepine receptors than those of the high TI (high fear) quail and that these receptors were more sensitive to GABA stimulation in the long TI than in the short TI line. GABA is a neurotransmitter. The benzodiazepine receptors are the same receptor sites that are bound by the tranquilizing drug valium (diazepam). These findings indicate that selecting birds for either short or long toxic immobility affects both the number and the affinity of neurotransmitter sites in the brain. The sites affected by selection for either high or low fear are bound by both tranquilizing drugs and brain neurotransmitters that have a calming effect. These studies are evidence that selecting for fearful behavior affects neurotransmitters that modulate fearfulness.

Jacquet and Launay (unpublished data) also demonstrated that there is a tendency for certain plasma cathecolomines to be more abundant in the brains of LSR line birds than in those of HSR line birds. However, none of the differences reported were statistically significant. Isolation induced similar increases in the plasma adrenaline level in both lines.

There has, therefore, been some effect of selection on brain metabolism but again more work needs to be done before it will be possible to elucidate the various mechanisms involved.

Conclusions

Selection for low fear and high social motivation seems likely to produce animals that show better adaptation and have better welfare under intensive husbandry conditions. Our experiments used Japanese quail; this species appears to be a good model of the domestic chicken, and it is likely that similar selection could be conducted in that species. Furthermore, fear and social

motivation are such basic motivational systems that with appropriate modification of the methods used, similar selection techniques could be used in a wide variety of domestic species.

Fear and social reinstatement behavior have general adaptive value. It is unlikely that fear reactions can be completely eliminated from any environment; all environments involve social reactions through either social behavior or social deprivation. The experiments described here show that selection for or against fear and sociality is possible using simple short-term measures and that the traits selected are not specific to a given age or environment.

The short tonic immobility line showed less fear and fewer fear-induced stress responses than did long tonic immobility line chicks in most of the situations investigated. They are therefore probably better adapted to intensive rearing conditions and would be likely to maintain greater productivity if subjected to environmental stress. High social reinstatement line chicks showed greater social affiliation in most of the situations tested. They would therefore probably be better able to adapt to high stocking densities.

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Chapter 12

Genetic influences on the behavior of chickens associated with welfare and productivity

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ABSTRACT
Many behaviors in poultry can be modified by genetic selection. Selection of laying hens for maximum egg production had the unfortunate side effect of increased rates of beak inflicted damage on other birds. Selective breeding has eliminated broodiness and has either increased or decreased other behaviors, such as hysteria, fearfulness, appetite in broilers, social dominance, ability, and damage to other birds. Genetic selection can be used to reduce behaviors that cause welfare problems. However, it must be approached with caution to avoid unintended consequences that would be detrimental to welfare. A calm, docile bird that appears behaviorally calm, may take longer for its heart rate to return to normal after it is frightened. The use of group selection instead of single-bird selection can be effectively used to reduce undesirable behaviors such as feather pecking and to maintain high egg production. An entire group of birds is selected instead of selecting individuals.

Preface to genetic influences on the behavior of chickens associated with welfare and productivity by Temple Grandin

The basic principle presented in this chapter is a comparison between selection for the most productive individuals vs selection for the most productive groups. Since its publication in the Second Edition in 2014, the principles of William Muir's work has drawn great attention in both the business and management press. This is due to the possible similarities between the behavior of people and the behavior of chickens. I found over 20 business trade publications that discussed his work and how it may be applied to management in big corporations.

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Muir's original work showed that when laying hens were selected based on the individuals with the highest egg production, this resulted in aggressive bullies who pecked and attacked the other hens. If small groups of hens was selected for the highest egg production for an entire group, the birds were highly productive and less likely to peck each other. In 2016 Dr. Muir was interviewed by David Sloan Wilson for Thisviewoflife.com. The title of the article was *When the Strong Outbreed the Weak: An Interview with William Muir*. In this interview, he explained that the group selection principle also applies to selecting pigs. When pigs were individually selected for lean meat and rapid growth, this resulted in aggressive pigs that attacked others. When group selection was used, the pigs were more docile and they seldom fought or bit tails. In my own work, I have observed both types of pigs. The differences between the two types of selection was obvious. The breeding company that used individual selection created excitable pigs that fought and bit tails. Another breeding company named Newsham used group selection. They produced pigs that were calmer, very productive, and seldom bit tails. There was one problem. They learned that calm group selected pigs must never be mixed with individually selected pigs. They will get attacked and injured. These principles may also apply to corporate management. Margaret Heffernan, a management consultant, was interviewed on The TED Radio Hour on NPR. She stated that selecting management employees based only on individual productivity may motivate them, to depend on the failure of others. This is an interesting idea that was inspired by Muir's research. Animal breeders have been slow to adopt the group selection approach. Maybe all the attention in the corporate world will make them reconsider their methods. Group selection may be one method for improving animal welfare in farmed animals.

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Introduction

Behavior is the observable actions, mannerisms, and means by which animals cope with their environment, including other animals in that environment. The environment may be considered in terms of both the internal or physiological environment and the external environment. The latter may be subdivided, with some overlapping, into social and physical aspects. When

failure to cope with changes is detected in chickens, the question arises as to whether there should be an attempt to adapt the environment to the bird or the bird to the environment (Muir, 1996, 2003; Muir and Cheng, 2004). Similarly, Cheng (2007) questions "Should we change housing to better accommodate the animal or change the animal to accommodate the housing?" Attention in this chapter is focused on genetic modification to improve the chickens to adaptability to their environments and consequently to enhance their welfare.

In the last half of the 20th century, the greatest change in the layer industry was that from floor pens to batteries of multiple-hen cages. The popularity of multiple-hen cages resulted in part because commercial producers found that multiple-hen cages offered several advantages in both economic and animal welfare aspects, including higher feed efficiencies (Cunningham, 1992), improvement of biosecurity and food safety (De Vylder et al., 2011; Hannah et al., 2011; Holt et al., 2011; Jansson et al., 2010; Jones et al., 2011), and reduced housing and labor costs (Craig, 1982; de Boer and Cornelissen, 2002; Sumner et al., 2011; Van Horne, 1996). Ironically, the biggest change in the 21st century may be away from cages and a return to floor pens or alternative rearing systems (California proposition 2, 2008; United Egg Producers UEP, 2011). Craig and Swanson (1994), Goodwin (2001), Mellor and Stafford (2001), Hemsworth (2007), Swanson (2008), and Mench et al. (2011) examined both historical and current ethical perspectives and attitudes toward husbandry conditions of livestock. Although there is considerable disparity among different social and professional groups, it appears that during the last three decades there has been a definite shift in the direction of greater concern in matters of pain and suffering of farm animals and a tendency to favor protectionist legislation (Mench, 2008; Mench et al., 2011; Millman et al., 2010). Public pressure for increasing the well-being of chickens in Western Europe led to codes of recommendations for "humane" husbandry and, in the extreme, legislation was enacted phasing out the use of conventional cages for layers starting in 2012 in the European Union (CEC, 1999) and some states of the United States (California proposition 2, 2008). It is of special interest that the prohibition of caging hens occurred even though cages have been shown to have some advantages over alternative housing systems (Appleby et al., 1992; de Wit, 1992; Lay et al., 2011; Mench et al., 2011), for example, cannibalism is more common in noncage systems than in dimly lit cage houses.

Behavioral problems exist not only among chickens kept in cages but also in alternative systems (Appleby and Hughes, 1991; Craig and Adams, 1984; Lay et al., 2011; Mench, 1992; Siegel, 1989). Recent reviews examined the future of the poultry industry and included issues related to politics, housing, ethical, and well-being concerns (Hester, 2005; Lay et al., 2011; Mench, 2008; Mench et al., 2011; Millman et al., 2010; Newberry and Tarazona, 2011) but perhaps the most striking conclusion given this move

away from cages was that of Lay and associates (Lay et al., 2011) who concluded that there is no one clear solution to the housing issue and that no housing system is ideal from a hen-welfare perspective. In floor pens and outdoor systems, hens are exposed to litter and soil, providing a greater opportunity for disease and parasites with similar consequences from adding environmental complexity. Further, the more complex the system, the more difficult it is to manage and with larger group sizes, disease is more likely to spread. Additionally, increasing the space per hen increases incidence of cannibalism and smothering and introduces difficulties in terms of disease and pest control while adding environmental complexity increases undesirable behaviors that are detrimental to welfare (Lay et al., 2011).

A promising resolution was suggested by Muir and associates (Muir, 1996, 2003; Muir and Cheng, 2004) and others (Cheng, 2007, 2010; Laible, 2009; Lay et al., 2011; Newman, 1994; Cheng and Muir, 2005) who concluded that selective breeding for desired traits may help to improve welfare in domesticated settings and decrease or eliminate instances of undesirable behaviors in cages or alternative housing systems. The focus of this chapter is toward changing the nature rather than the nurture of chickens to improve welfare in domestic environments.

Assessment of welfare

The words welfare and well-being are widely used but connotations vary. Dictionary definitions reflect an anthropomorphic viewpoint, for example, Merriam-Webster, Inc. (1983) defines welfare as "the state of doing well esp. in respect to good fortune, happiness, well-being, or prosperity" and well-being is defined as "the state of being happy, healthy, or prosperous." To animal researchers, prosperity and happiness are unlikely to be meant, but rather absence of chronic pain and suffering, good health, ability to respond to stressors, and components of fitness (including productivity in some contexts) are likely to be included. There is a tendency, especially among Western Europeans, to place emphasis on mental conditions such as fear, frustration, anxiety, anger, and similar emotional states (Barnett and Hemsworth, 2003; Duncan and Dawkins, 1983; Duncan and Petherick, 1991; Green and Mellor, 2011; Mellor, 2012; Yeates and Main, 2008). Although most agree that emotional states should be included, it needs to be recognized that certain temporary stressors, likely to be associated with unpleasant mental states, prepare chickens to cope better with more severe stressors later in life (Gross, 1983; Zulkifli and Siegel, 1995). Life in general is a series of stressors, for humans and animals, in the quest for food, shelter, security, and reproduction. In each of these basic quests, even in natural conditions, most animals experience periods of food deprivation, disease, predation, competition, and stress. Our quest should not be to provide as natural conditions as possible, lest we expose animals to the undesirable aspects of nature

as well, but rather to protect animals from such natural stressors, while ensuring a safe healthy food supply, free of disease, for human consumption.

Criteria of welfare

Having defined, in very general terms, what is meant by well-being, it is then necessary to examine how it may be evaluated. In what follows we adopt the general categories of evidence suggested in the review of Craig and Adams (1984). More detailed descriptions may be found in other reviews (Craig and Swanson, 1994; Duncan, 2005; Mench, 1992; Webster, 2001; Yeates and Main, 2008).

Overt signs

Bodily injury, morbidity, extreme weight loss, heavy feather loss (not associated with natural molting), and death of individuals are widely accepted as indicators that welfare is impaired. When the well-being of groups in production settings is considered, longevity, that is, days' survival over the production period, can be used as an overt indicator. This measure takes into account all aspects of stress (Hurnik, 1990; Muir and Craig, 1998; Quinteiro-Filho et al., 2012). Lifetime stress as the result of adverse environments will increase susceptibility to diseases and will result in other physiological manifestations which shorten the life-span. Vices such as feather and cannibalistic pecking also increase exposure to disease or cause death. Thus, longevity, if measured over a long period, is a direct measure of welfare.

Physiological and immune responses

The general adaptation syndrome (GAS) can be delineated into short-term fight or flight responses and longer duration accommodation responses involving the hypothalamic–pituitary–adrenal (HPA) and the sympathetic–medullary–adrenal axes and is associated with immunocompetence and health. Siegel (1981) stated that, based on general metabolic effects ascribed to stress, there is a direct link between stress and susceptibility to disease and that social stress is an effective initiator of nonspecific stress response in fowl. Increases in social stress reduce antibody levels and thereby lower the resistance of fowl to several important viral infections and to bacterial infections of the respiratory system. Nonspecific environmental stressors can have significant influences on growth, reproduction, and the ability of the bird to resist diseases (Siegel, 1981). Dantzer (1994) indicated that animal response to stressor by changing neurohormonal homeostasis does not depend on the physical nature of the stressor. Corticosteroids, as a final compound, are produced in response to stressors. These produce many of the symptoms associated with long-term stress, including cardiovascular disease, hypercholesteremia, and modifications of the immune function (Siegel, 1995).

Although physiological (including immune) responses frequently reflect the presence of known stressors (Freeman, 1985; Gross, 1983; Hill, 1983; Hoehn-Saric and McLeod, 2000; Piazza et al., 2010; Siegel, 1993; Theorell, 2012; Zulkifli and Siegel, 1995), it has been shown also that such measures can occasionally be misleading or difficult to interpret. For example, plasma corticosteroid concentrations were found to be elevated similarly for hens in high-density cages and spacious floor pens even though other measures indicated that those in high-density cages were under greater stress (Craig and Craig, 1985; Craig et al., 1986).

Functional genomics

At a recent Poultry Science Keynote Symposium—Tomorrow's Poultry: Genomics, Physiology, and Well-Being (Muir et al., 2010), a novel approach to objectively quantify stress and well-being in animals and to identify management practices that necessarily compromise well-being was discussed. Toward that goal we proposed using the RNA transcriptome as a holistic tool to formulate a metric. The transcriptome represents the expression of all genes in an organism at a given time in response to environmental stimuli. The output from each gene can be quantified on a microarray, or more recently using whole-genome sequencing techniques, commonly termed RNAseq. The methodology for developing this technique was described in a landmark paper by Golub et al. (1999) and recently further reviewed by Myers (2011) and Klopfleisch et al. (2012). They demonstrated the power of supervised clustering to diagnose cancer type in humans. In the first step cancer types were classified by a panel of experts. In the second step the transcriptome was used in a discriminate analysis to predict each cluster. In the third step the transcriptome was sampled on a new set of individuals, and the samples assigned to clusters based on the discriminate function in the previous step. The results were verified by the same team of experts and were found to be extremely accurate. Knowledge of which genes were up- and down-regulated by each cancer type was not needed, only the pattern was important. Transcritomic profiling has also been used widely in improving human well-being (Chaussabel et al., 2010; Elstner and Turnbull, 2012; Hindmarch and Murphy, 2010; Mestan et al., 2011).

Similarly, application of this novel approach for assessing well-being has the potential to provide common ground and address housing and other issues allowing the animals to speak for themselves through their transcriptome. The approach would include known stressors and animal-friendly environments. Exposure of the animals to those environments for short- and long-term durations, and then collection of their transcriptome from tissues in which emotions are known to be controlled, such as the hippocampus and the HPA axis. The transcriptome would then be isolated and quantified, using the latest technology, such as RNAseq. A supervised cluster analysis would then be performed into

known treatments to create a discriminate function. The discriminate function classifies observations by the metric used to create the function. Next, an independent set of animals would be exposed to contentious alternative housing and management practices for short- and long-term duration, the same tissues isolated, the transcriptome quantified, and finally clustered using the previously trained discriminate function. The function will classify questionable housing and management practices into those that are most similar to those recognized as stressors and those that are animal friendly.

Alternatively, if training environments cannot be agreed on by a panel of experts, an unsupervised clustering analysis can be performed on the transcriptome of animals housed in alternative and questionable environments. An unsupervised cluster analysis will at least show if the alternative environments are really different *as perceived by the animals* in the environmental conditions imposed for whatever reason. If two environments do not induce a different transcriptome profile, it would be difficult to argue that the environments are inducing different levels of stress.

Productivity

The use of productivity or performance data as indexes of well-being in chickens has been criticized (Hill, 1983). However, as Craig and Adams (1984) made clear, early comparisons confounded profitability and productivity. When productivity of the individual laying hen is considered in terms of number of eggs, feed efficiency, and mortality, it proves to be a fairly sensitive indicator of well-being (Adams and Craig, 1985; Appleby and Hughes, 1991; Cunningham et al., 1988). Productivity indicators may be useful measures of the continuing effects of stressors when hens have made an adjustment to the "stage of resistance" as defined by physiological criteria. For example, Beuving and Vonder (1978) reported and reviewed by Ruszler (1998): little or no behavior evidence of the stressful conditions of high temperature or water and feed deprivation after the first few days, though their hens stopped laying.

The relationship between stress and production also supports the notion that production is a good indicator of stress. Siegel (1995) and Scheele (1997) pointed out that stress has important consequences on the birds' well-being, especially those that affect energy and mineral metabolism and interactions with the immune system. Social or behavioral environments can also activate stress responses in birds just as physical stressors do (Cheng et al., 2002, 2003). Results may manifest themselves in reduced growth in juveniles, reproductive capacity in adults and increased food consumption.

When management of broiler breeder stock is considered, a problem with interpretation of their welfare arises. Because of excessive appetites, birds to be kept as broiler breeders are severely restricted in feed intake. This practice prevents obesity of adults which if uncontrolled reduces health,

longevity, and reproductive performance, and increases the incidence of leg problems (de Jong et al., 2005; Hocking et al., 2004; Siegel, 1984; Tolkamp et al., 2005). By the criteria cited above, productivity indicates improved well-being with broiler breeders on feed restriction. Nevertheless, feed deprivation has been shown to cause increased aggressive pecking of chickens (Duncan and Wood-Gush, 1971; King, 1965; Mench et al., 1986; Shea et al., 1990). Nevertheless, from the standpoint of physiological indicators, it appears that broiler breeders on feed restriction adjust rapidly (Benyi et al., 2009; Katanbaf et al., 1989).

Behavior

European researchers often use behavioral criteria as the primary, or sometimes the only, indicator of hens' welfare (Baxter, 1994; Faure and Mills, 1989; Savory and Hughes, 2010; Sorensen, 1981; Wegner, 1985). Dawkins (1983) was critical of such an approach and wrote:

At first sight, the growing awareness that an animal's behavior may be important evidence to have when assessing its welfare is entirely to be welcomed. But there is, amid the encouraging trend of a greater use of ethological data, also a disturbing one of possible misuse of ethology.

For example, Dawkins (1988) considered the absence of behavior patterns seen in the wild as an invalid criterion of animal suffering although such evidence is frequently used by critics of high-density cage environments.

Assessment of chronic pain by behavioral criteria appears to be a valuable technique. For example, behavioral evidence suggested that pain and heightened beak sensitivity persist for several weeks or even months following beak trimming in hens trimmed at 5 weeks of age or older (Breward and Gentle, 1985; Craig and Lee, 1990; Duncan et al., 1989; Gentle, 1986b, 1989, 1992; Gentle et al., 1990, 1991; Lee and Craig, 1990, 1991; Mench and Duncan, 1998). Nevertheless, interpretations of behavioral evidence can be controversial. Thus Eskeland (1981) observed that beak-trimmed hens spent more time "resting" and concluded that fearful running by hens of low social rank was almost eliminated by beak trimming. However, Gentle (1986a) suggested that the significant increase in resting behavior among trimmed-beak birds was similar to the inactivity following injury seen in both human beings and animals (Cheng, 2005; Wall, 1979). Because of interpretation difficulties, it is valuable to supplement behavioral observations with other types of evidence as was done by Breward and Gentle (1985) and Gentle (1986a, 1989). They presented evidence that following partial beak amputation, growth of neuromas occurred in the part of the beak remaining and that spontaneous firing of afferent neurons associated with pain occurred up to at least 12 weeks after beaks were trimmed.

Several researchers, including Dawkins (1983) and Faure (1986), have used the willingness of hens to express preferences or "work" for

environmental conditions allowing the expression of certain behaviors as evidence of the relative desirability of alternative environments from the hen's viewpoint. Nevertheless, possible confounding factors must be avoided or compensated for in such tests because previous experience with an environment may have a powerful effect on motivation to choose or work for it so that birds with different experiences choose differently (Byholm and Kekkonen, 2008; Campos et al., 2008; Cornil and Ball, 2010; Mumme et al., 2006; Petherick, et al., 1990).

Behaviors seen as indicating problems with the environment, such as vacuum activities and stereotypies, are frequently cited as indicators that the environment impairs welfare in animals (Mason et al., 2007; Martins et al., 2012). However, Hughes (1980), Dantzer (1993), and Mason and Latham (2004) argue that the occurrence of such activities may allow animals to cope.

General

Difficulties of interpretation of the various criteria of welfare have been discussed (Clark et al., 1997; Consortium, 1988; Craig and Adams, 1984; Craig and Swanson, 1994; Dawkins, 1998; Hetts, 1991; Hill, 1983; Hurnik, 1990; Izzo et al., 2011; Kumar et al., 2012; Mench, 1992; Muir and Craig, 1998), and there is a tendency to agree that, except for overt indicators, no single measure has previously been shown to be adequate, at least when long-term stressors are involved. Therefore so far as possible, multiple indicators are preferable. One such multiple indicator is the transcriptome, which includes all expressed genes in the genome and thus includes all physiological responses to the environment.

Domestication and behavior

Behaviors associated with fitness in a natural environment may not differ greatly from those found in domesticated stock under relatively primitive husbandry conditions. Thus "native fowl" in villages of Southeastern Asia obviously cope with their environment although they may receive only minimal care such as being fed unusable by-products of subsistence farming or leftover food scraps and some protection from predators.

Those behaviors favoring domestication of a species have been described (Cheng, 2010; Hale, 1969), and chickens fit the pattern well. Behaviors that were essential in the wild may be unnecessary or even counterproductive in terms of well-being under modern systems of husbandry. Thus extreme alertness and flighty behavior may lead to waste of energy and injuries in attempting to escape from otherwise harmless stimuli when predators are not present. Aggressiveness in obtaining desired resources may result in low-status birds' failure to receive adequate necessities even when enough is supplied for all. Broody behavior, essential to fitness in natural environments, is

undesirable in domestic conditions and has been all but eliminated by breeders in egg-production stocks.

Genetic versus environmental influences

As species-specific activity, behavior is under genetic control through regulating physiological homeostasis. In artificial environments, it may be difficult to know, in the absence of experimental data, whether differences between stocks are of genetic or environmental origin or if both kinds of influence are at work. For example, age at onset of egg laying, egg weight, intensity of egg production, and duration of laying have been under effective genetic selection for many generations but the stimulus of day length and artificial control of photoperiods can also have large effects. Attempts have been made to rule out environmental effects by standardizing environmental conditions, as when stocks are compared for egg production in random sample tests. Even so, stocks being compared may have different requirements for maximum performance or special characteristics which make them superior in some environments and inferior in others.

The existence of genotype by environment interactions for behavioral traits (as for production traits also) complicates the assessment of behavioral problems and possible approaches for improving the welfare of chickens by genetic means. Thus, if a particular egg-laying stock should possess a limited appetite and therefore require a more concentrated ration for optimal well-being and productivity, it would be penalized if compared with other stocks having greater appetites if all are fed a ration more suitable for the latter stocks.

Behavioral differences among populations

Behavioral differences, usually of a quantitative nature, are found among domesticated stocks of chickens. Most of these can be demonstrated to be of genetic origin. Often it is difficult or impossible to know how these differences arose. Likely contributors consist of genetic differences in foundation populations, random genetic drift in relatively small and isolated populations, adaptation to local environments by natural selection, and different goals under artificial selection. The impact of selection by poultry breeders is clear when comparing breeds developed specifically for beauty, fighting ability, meat production, or table-egg production. As an example of how selection for an economic trait can affect behaviors, Siegel (1989) described how long-term selection for heavy juvenile body weights in broilers resulted in their having more docile behavior, greater appetite, and reduced motor activity when compared with egg-production stock.

Comparisons among stocks are complicated in some cases because of developmental stage effects. Studies by Tindell and Craig (1959) and Bellah

(1957) are especially instructive in this regard. Tindell and Craig found differences in peck-order status among hens of four breeds (White Leghorn, Australorp, Rhode Island Red, and White Plymouth Rock) when placed together in intermingled flocks and observed to determine relative peck-order status at about 5 months of age. Bellah used hens of the four breeds from the same experiment that had been kept in separated-strain flocks and determined their relative social dominance ability beginning at about 8 months of age. Strikingly different results were obtained from those of Tindell and Craig when relative social dominance ability of the breeds was compared at the later age. In the most extreme case, Rhode Island Red hens, which ranked lowest among the breeds when compared in intermingled flocks at 5 months, were the most dominant when tested in pair contests at 8 months of age.

Inconsistencies have also been found for social dominance ability among experimental stocks within the White Leghorn breed when compared at different ages. Using two populations subjected to long-term selection for number of eggs laid before 40 weeks of age, mostly in competitive floor-pen environments, and the unselected control from which they were derived, Craig et al. (1975) found that the selected stocks had decreased age at sexual maturity and increased aggressiveness and social dominance ability during the adolescent period. However, for one of the selected strains, rank for the agonistic traits was reversed relative to the control stock between adolescence and full maturity.

Social inertia can confound accurate determinations of relative social dominance ability after peck orders have become well established (Guhl, 1968). Lee and Craig (1981a) demonstrated this using the same stocks compared in the Craig et al. (1975) study. They found that the selected strain had social dominance over the control at 5 months (before egg laying), but the selected stock dominance ability had diminished or disappeared when strangers of the strains were placed together at 17 months of age. Nevertheless, selected strain pullets maintained undiminished dominance over control pullets for the entire laying year when they were kept together from time of housing at 5 months.

Comparisons among breeds, in which a single stock within each breed was used, have revealed behavioral differences of genetic origin. Such differences were found for chicks' open-field activity (Faure, 1979), presence or absence of hysteria among hens in large-group-size cages (Elmslie et al., 1966), duration of tonic immobility (Campo and Alvarez, 1991; Campo et al., 2007; Gallup et al., 1976; Jones and Faure, 1981), and prelaying behavior of individually caged hens (Mills and Wood-Gush, 1985), and eating (Howie et al., 2011). Such studies are of value in demonstrating whether genetic stock differences exist at all but could be misleading in terms of identifying breeds as having particular characteristics. For example, large strain differences have been shown within the White Leghorn breed for

behavioral traits. Thus, Hansen (1976) found hysteria-susceptible and hysteria-resistant strains. Al-Rawi et al. (1976) and Buitenhuis et al. (2009) detected differences in frequency of agonistic acts; and hens of different stocks were found to differ in feather-loss and mortality from cannibalism when their beaks were left intact (Craig and Lee, 1990).

Inbreeding depression and random genetic drift

Expected general consequences of inbreeding within a population are: there will be subdivision into distinctive lines or subpopulations (because of random genetic drift), uniformity will increase within subpopulations, and there will be a general loss of vigor. Although there are widespread observations over species confirming the consequences of inbreeding in general, the evidence for behavioral traits in chickens is sparse.

A study by Craig and Baruth (1965) examined the effects of inbreeding on vigor as indicated by social dominance ability of cockerels and pullets. Because of small numbers available within each of five partially inbred lines, differences among lines and uniformity within lines were not examined. However, mean performance over the lines when compared with that of a sample of the noninbred and unselected population from which the lines were derived provided the evidence needed. Partially inbred cockerels were inferior in ability to win pair contests when matched with noninbreds, and pullets' peck-order status was negatively associated with their inbreeding coefficients.

McGibbon (1976) observed that the incidence of floor laying in pens increased as inbreeding increased in lines originating from random-bred populations of White Leghorns (Cornell Control) and Rhode Island Reds (Regional Reds). This evidence suggests that homozygous recessive genes were involved. However, environmental conditions during the rearing period were also identified as being influential; floor laying was more prevalent among confinement-reared pullets than in range-reared pullets.

Differentiation associated with keeping relatively small subpopulations isolated over several generations was illustrated for fear-related escape and avoidance behavior by Craig et al. (1983). One of two subpopulations selected alike required about 10-times as long to recover from fear as did the other (Craig et al., 1983). Likewise, differences in "righting time" following the induction of tonic immobility were present within some pairs of similarly selected lines (Craig et al., 1984).

Mendelian traits

Genes having major effects on morphology and the nervous system are typically deleterious in terms of the well-being of affected individuals and often affect their behavior (Axelsson and Ellegren, 2009; Eyre-Walker and

Keightley, 2009; Siegel and Dunnington, 1990). Such genes are usually either sex-linked recessives, which may be expressed in noninbred stocks, or autosomal recessives, most likely to be seen following inbreeding. Because of the generally deleterious effects of these major genes they are selected against and are not seen in commercial stocks except at very low frequency for homozygous recessive phenotypes.

Some major genes, seemingly having adverse effects, may not be as disadvantageous as they seem at first consideration. For example, results of a study carried out by Ali and Cheng (1985) are relevant. They compared genetically blind hens (rc/rc) and sighted hens (Rc/rc) segregating from matings of Rc/rc × rc/rc chickens. During a 2-month experimental period, blind hens were less active, had better feather cover, produced more eggs, required less feed, and did not differ in body weight from sighted hens.

Selection involving behavioral traits

Behaviors associated with welfare and productivity in commercial stocks are typically under polygenic control and are measured in quantitative terms. However, some, such as "broody" behavior, are more of an all-or-none nature. Both autosomal and sex-linked genes may be involved. Because selection studies are costly and time consuming, breeders often depend on heritability estimates in guiding choices as to whether selection is likely to change behaviors of interest. Heritability estimates often have large standard errors and are most relevant to the population in which the data for their estimation are gathered.

Effects of both direct and indirect (genetically correlated) selection responses on several relevant behaviors have been carried out and will be reviewed below. Although selection to improve behaviors associated with well-being has been effective in most experimental studies, commercial breeders are under economic constraints that may prevent their adoption of programs in which behavioral problems are addressed (Faure, 1980, 1981a; Faure et al., 1992; Hocking, 1994). Nevertheless, results of some recent studies (Cheng, 2010; Cheng and Muir, 2005, 2007; Kuo et al., 1991; Muir, 1996) show that major advances may be made within a reasonable number of generations in reducing behavioral problems having an economic impact.

Some behaviors are easily identified and therefore amenable to selection procedures, as in the case of broodiness, or because they leave "traces" (Faure, 1981b), as in the case of feeding behavior which can be measured from feed consumed. However, as Faure (1981b) and Hocking (1994) indicated, selection involving traits such as prelaying pacing (see below) must be measured by laborious and time-consuming observations that are likely to be impractical for breeders who must evaluate hundreds or thousands of birds. Also, breeders know that each additional trait added to a breeding program reduces the effectiveness of selection for other traits of importance.

Broodiness

Behavior related to incubation of eggs is no longer evident or occurs with very low frequency in major commercial stocks in which broodiness has been selected against. Incubation behavior of hens was a common characteristic in most chicken stocks until a few decades ago. As the need for natural incubation and maternal care of chicks became obsolete, because of artificial incubators and chick brooding equipment, broodiness came under closer scrutiny.

Broodiness is a trait involving sex-linked genes as suggested by Warren (1930) and confirmed by later studies (Dunn et al., 1998; Jiang et al., 2005; Ohkubo et al., 1998; Saeki, 1957). Early on it became evident that within the White Leghorn breed incidence was relatively low whereas other breeds usually had higher levels. Although Saeki (1957) obtained estimates of heritability of less than 20% in the Japanese Nagoya fowl, an early selection study by Goodale et al. (1920) indicated that realized selection progress was substantial in the Rhode Island Red stock used. The fact that broodiness was successfully selected against by commercial breeders is not surprising; it appears to be at least moderately heritable (Romanov et al., 1999); most experiments indicated that it is negatively associated with total egg production; and it is easily identified during the first year of egg production (Saeki, 1957).

Should there be concern about the loss of a previously important behavior from chickens? We believe the absence of a behavior previously expressed is weak evidence that suffering results. Craig and Swanson (1994) discussed this as follows:

With the example of broodiness in mind, one may ask, why should behaviors that are no longer required be expressed? Presumably, most or all such behaviors could be eliminated by genetic selection between or within stocks, so that hens would not be motivated to show them or be frustrated by the absence of conditions allowing their expression.

Behavioral exhibition is controlled by both internal and external factors. Internal factors are mainly neuroendocrine factors. Neurons of the central nervous system have the capacity to change structure and functions (neuronal plasticity) to adapt to a given environment (Joseph, 1999; Kolb and Whishaw, 1998; Smith, 1993), which, in turn, affects animals' behavioral exhibition (Rosenzweig and Bennett, 1996; Sausa et al., 2000). Through domestication, farm animals are quite different from their wild counterparts in many ways, including behavior. In addition, the degree of welfare could be dramatically different between strains of a species and individuals of the same strain even housed at the same environment, which is dependent on each individual's behavioral and physiological characteristics (genotypic and phenotypic variations). Adaptation of an individual, but not another, to its surrounding environment can be achieved through environmental

stimulation-associated behavioral and physiological plasticity in the animal (a genetic−environmental interaction) and the experiences received during the animal's lifetime (allostasis, i.e., allostatic load or overload) (Clark et al., 2007; McEwen, 2001).

Stereotyped pacing during the prelaying period

Breeds, strains within breeds, and individuals within strains vary in stereotyped prelaying pacing seen in single-hen cages. Such behavior may be interpreted alternatively as indicating frustration (Duncan and Wood-Gush, 1972) or the alleviation of stress (Hughes, 1980; Dantzer, 1993). The absence of nests in single-hen cages is apparently responsible for stereotyped pacing before an egg is laid in some stocks but not in others (Mills and Wood-Gush, 1985). Instead of pacing, some stocks show quiet sitting behavior during the prelaying period. Thus, it may be conjectured that those stocks showing pacing are frustrated whereas other stocks are not. If convincing evidence becomes available that well-being is compromised in stocks that pace in the absence of nests, then choices need to be made. For example, either cages should be modified by providing nests, stocks should be used that are not frustrated by the absence of nests, or within-strain selection should be directed toward selecting for quiet sitting behavior rather than stereotyped prelaying behavior. Mills et al. (1985) demonstrated, in a brief selection study, that such selection could be effective.

Increased pacing before egg laying was not seen in high-density, multiple-bird cages, as shown by Ramos and Craig (1988). Crowding in multiple-hen cages reduces the opportunity for increased pacing behavior. Even so, the question arises as to whether the environment in crowded cages differs enough from that in single cages so that results obtained in single-bird cages are relevant. The greater "enclosure" of space which exists among hens in a group may result in less anxiety than would be seen in an individual-hen cage. Therefore, the question arises as to whether selection against stereotyped prelaying pacing, even if found to reduce stress in that environment, would also reduce stress in multiple-hen environments.

Feather and cannibalistic pecking

A behavioral problem occurring especially among hens kept for table-egg production is feather loss and cannibalism. In most production settings these vices are greatly reduced by beak-trimming. However, behavioral and physiological evidence, reviewed earlier, indicated that beak-trimming causes pain which persists for weeks and perhaps even months. The Brambell committee (Bramble, 1965) concluded with reluctance that de-beaking of birds should be permitted for a limited time to control outbreaks of "vice" (feather pecking and cannibalism). However, within a short period of time they hoped

that suitable strains would be available and in adequate supply so that debeaking was no longer necessary.

Craig and Muir (1993) showed that direct selection to reduce beak-inflicted injuries could be rapid when group selection was used. They kept intact-beak hens separately by sire families in multiple-hen cages and selected sire families to produce succeeding generations on the basis of hen-days without beak-inflicted injuries for 168 days from 16 to 40 weeks of age. Selected lines achieved 75% of the possible selection differential by the second generation.

Agonistic behavior

Gamecock matches frequently terminate in the death of the loser. Selection of winners in game breeds to sire progeny of future generations has without doubt included selection for strength, agility, stamina, and other elements of behavior necessary for that outcome. Game breeds also have accelerated blood prothrombin and coagulation times (Mohapatra and Siegel, 1969) and arteries and veins that are stronger, smaller in diameter, and thicker walled as compared with White Rocks (Steeves and Siegel, 1968). Social dominance of males was strongly associated with fitness in small flocks of nongame breeds (Guhl and Warren, 1946). However, in somewhat larger flocks with higher densities, Kratzer and Craig (1980) found only a moderate correlation of male social status and completed matings. With even larger flocks and sex ratios similar to those used in commercial breeding flocks, Craig et al. (1977) found that social status of White Leghorn cockerels appeared to have little effect on frequency of mating.

Bidirectional selection for high and low social dominance ability, based primarily on the outcomes of initial pair contests in both sexes, was carried out over four generations in White Leghorns by Guhl et al. (1960). In a similar study Craig et al. (1965) selected on the basis of pair contests between males only over five generations within White Leghorns and Rhode Island Reds. Although realized heritabilities were only moderate, very large differences between high and low strains were produced by the end of the studies in strains derived from each of the three foundation stocks. In the more comprehensive study of Craig et al. (1965), there was no obvious decrease in additive genetic variation over generations for the selected trait. The availability of unselected random breeds from the foundation stocks and testing of both males and females by Craig et al. (1965) revealed that the responses were essentially symmetrical and were not sex specific. Peck-order status of selected strain hens in intermingled-strain flocks within breeds differed significantly, but the differences appeared to be of reduced magnitude as compared with relative status based on initial pair contests.

McBride (1962), noting the results of Tindell and Craig (1959), postulated that high aggressiveness in a genetic stock would have the same effect

on productivity as he had hypothesized for poor husbandry (McBride, 1960). Specifically, he predicted that more aggressive strains would be under greater stress and would have lower mean performance levels and greater variances. Further, he postulated that strains with higher levels of aggressiveness would have a greater negative skew to their frequency distribution curves for productivity traits than found in strains having lower aggressiveness (McBride, 1968).

McBride's hypothesis was tested by Craig and Toth (1969) using hens of the high and low social dominance strains from the White Leghorn and Rhode Island Red strains developed by Craig et al. (1965). Strains were kept separately in floor pens and social instability was assured in half of the pens of each strain by randomly redistributing birds among flocks weekly. Although the unstable flocks had more fights, peck-avoidances, and threat-avoidances than the stable flocks (Craig et al., 1969), there was no evidence of loss of productivity in the unstable flocks. A possible explanation advanced was that the greater frequency of agonistic interactions in unstable flocks were not necessarily associated with greater stress for all hens because the frequent changes in group membership would benefit those individuals that otherwise would have been low in social status over the entire test period if they had been kept in stable organizations.

Mating behavior of males

Two bidirectional selection studies for frequency of matings by males (Tindell and Arze, 1965; Wood-Gush, 1960) indicated that large differences could be produced readily. However, a long-term bidirectional study, in which the foundation population was maintained as a control, by Siegel and his colleagues (Dunnington and Siegel, 1983) indicated complex results. It became evident early in the study that results were not symmetrical. Although the initial response to selection in the high line was near zero in the first few generations, responses then began and accumulated until at least 20 generations had elapsed. Realized heritability was 0.18 when calculated over 23 generations. In the low line very different results were obtained; a realized heritability of 0.32 was obtained through generation 11 (Siegel, 1972), but thereafter a very large additional response appeared to be associated with little change in effective selection differentials (Dunnington and Siegel, 1983). Some males failed to mate at all during the test situation. The percentage of nonmaters decreased in the high line, was unchanged in the control, and increased dramatically in the low line. During generations 21, 22, and 23 the incidence was 84%, 89%, and 60%, respectively, in the low line.

A surprising lack of correlated responses occurred in Siegel's long-term selection study and an important genotype by rearing interaction was found. Although very large differences were present among the high, control, and

low lines for the trait of selection (Dunnington and Siegel, 1983), fertility in natural matings did not differ when males were present for extended periods of time or when artificial insemination was practiced (Bernon and Siegel, 1981). Females of the lines in generations 16 through 23 were tested for mating behavior, and when analyses were carried out within generations no consistent line differences were found (Dunnington and Siegel, 1983).

The question of whether males of the high-, control-, and low-mating frequency lines would respond similarly if reared with females as compared to separate sex rearing after 6 weeks of age was answered by Cook and Siegel (1974). A large genotype by rearing environment interaction was found. Rearing males of the unselected and low lines in heterosexual flocks increased the frequency of maters by about 20% whereas essentially all males of the high line mated regardless of how they were raised.

Appetite

From a controlled five-generation study of single-trait selection on body-weight gain, food consumption, and food conversion ratio (Pym and Nicholls, 1979) it was learned that all three selected lines responded with realized heritability estimates of 0.37, 0.44, and 0.27, respectively. Of particular interest from the behavioral standpoint is that food consumption and weight gain had a realized genetic correlation of 0.71. In a further study, the similar results were reported from their 10 generations of chickens (Pym et al., 1984). Therefore, selection for weight gain in broilers also involves considerable selection for appetite and vice versa. Food consumption records, collected in addition to body weight information, involve considerable expense in data collection but would allow selection for the economic trait of food-conversion ratio also. Presumably because of the extra expense involved and the fact that body weight and food-conversion ratio are correlated in the desired direction (−0.40 in the Pym and Nicholls study), commercial breeders have chosen to select for increased gains in body weight only. Obviously such selection has resulted in increased appetite in broiler stocks. From the considerations above, it comes as no surprise to learn (Nir et al., 1978) that in comparisons of a light and heavy breed to 18 days of age, heavy breed chicks consumed only 11% more feed and failed to gain more weight than ad libitum fed controls of their own breed when force-fed to gut capacity as compared to light-breed chicks that consumed 43% more feed and gained 30% more body weight when force-fed to capacity as compared to ad libitum fed controls of their breed. Because of their huge appetites, meat-strain birds kept as broiler breeders are severely restricted in feed intake to prevent obesity of adults which would otherwise reduce health, longevity, and reproductive performance, and increase the incidence of leg problems (Decuypere et al., 2010; Sandilands et al., 2005; Siegel, 1984; Su et al., 1999; Yu et al., 1992).

Profound effects of selection for food intake and for body weight to a given age in changing appetite and associated physiological phenomena have been demonstrated by several investigators, and especially by Siegel and his colleagues (Siegel and Dunnington, 1990). The latter selected bidirectionally for 8-week body weight over more than a quarter-century. Their high and low lines diverged dramatically not only for body weight (Dunnington and Siegel, 1985) but also for specific aspects of food intake behavior (Dunnington et al., 1987). Long-term selection for increased body weight apparently resulted in "genetic lesions" of brain satiety centers whereas selection for decreased body weight was offset by electrolytic lesioning of the satiety centers (Burkhart et al., 1983), respectively, involving the melanocortin circuit of the brain (Hen et al., 2006).

Because of poor appetite and associated low feed intake, minimal body weights and fatness required for the onset of egg laying may not be met (Bornstein et al., 1984; Zelenka et al., 1988). In the low-weight line described above, some females are anorexic, eating so little feed ad libitum that onset of lay is delayed or prevented (Dunnington et al., 1984); thus over 50% of pullets did not mature in generations 25 and 26 (Siegel and Dunnington, 1987). Zelenka et al. (1988) were able to induce egg production in nonlayers of this line by force feeding.

Walking problems and tibial dyschondroplasia

Tibial dyschondroplasia (TD), involving abnormal cartilage development in the proximal end of the tibia, occurs primarily in broiler chickens being fed ad libitum and the incidence is higher in males than in females. TD seriously impairs walking ability when present in advanced stages and may reduce weight gains due to the inability of birds to move freely in obtaining feed and water (Yalcin et al., 1995; Whitehead, 1997; Farquharson and Jefferies, 2000; Leach and Monsonego-Ornan, 2007). It has been shown that susceptibility is genetically influenced and that selection procedures are effective in separating lines selected bidirectionally for incidence (Leach and Nesheim, 1965, 1972; Ray et al., 2006; Sheridan et al., 1978; Shirley et al., 2003; Wong-Valle et al., 1993a). Wong-Valle et al. (1993a) included an unselected control in their selection study and after seven generations of selection incidences at 7 weeks of age were 92.0%, 5.4%, and 13.2% in the high selected, low selected, and control lines, respectively.

Primary breeders are concerned about the presence of TD in their stocks as indicated by their use of an instrument which quickly and accurately diagnoses the disease in live broilers (Bartels et al., 1989; Wong-Valle et al., 1993a). Deleterious effects of the disease among prospective broiler breeders are minimized by weight-control management. Although results are not always consistent among studies, it appears from the selection study that body weight at 7 weeks did not differ between the high- and low-incidence

lines in three of the first four generations (Wong-Valle et al., 1993b). Kuhlers and McDaniel (1996) and Zhang et al. (1998), using data from seven generations of selection in the same stocks, indicated that TD expression and body weights at 4 and 7 weeks of age are genetically and phenotypically independent traits.

Fear-associated behavior

Fear is an emotional internal state which can cause physiological indicators of stress (Bronson and Eleftheriou, 1965; Lang and McTeague, 2009; Ohmura and Yoshioka, 2009; Selye, 1956). Reliable behavioral indicators of fear are difficult to establish. For example, Duncan and Filshie (1979) showed that genetic stocks that appeared to be less fearful as indicated by escape and avoidance behavior, exhibited as much fearfulness as another stock when heart rate was used as the criterion of fearfulness. Also, different behavioral indicators may yield inconsistent results when genetic stocks are compared. Murphy (1978) outlined problems in classifying genetic stocks as "flighty" and "docile." Difficulties in classification of relative fearfulness were also encountered by Craig et al. (1983, 1984, 1986); who found that duration of tonic immobility as a criterion of fearfulness of genetic stocks gave results that were inconsistent with those obtained when the same stocks were compared in terms of escape and avoidance behavior. Therefore, caution is appropriate in interpretation of behaviors associated with fearfulness as indicating welfare problems unless other kinds of evidence are also present.

Nervousness, feather loss, and hysteria are likely to occur in some genetic stocks, but not in others, when group size is "large" and hens have been kept for a period of several months in barren, high-density cages (Buijs et al., 2009; Craig et al., 1983; Lay et al., 2011 Elmslie et al., 1966; Hansen, 1976; Shimmura et al., 2010;). Minimum group size associated with hysteria in susceptible stocks probably varies but the risk appears to rise rapidly when more than 12 are present. Repeated and severe episodes of hysteria are associated with reduced well-being because of resulting scratches, torn skin, feather loss, and reduction in feed consumption and egg production (Campo et al., 2001; Hansen, 1976).

Open-field activity

Open-field activity has been used as a criterion of fearfulness in rodents (Bellavite et al., 2009; Overstreet, 2012). The test in livestock has been recently reviewed by Forkman et al. (2007). Faure (1981a) selected bidirectionally for differences in open-field activity of 2- and 3-day-old chicks. The technique used was highly automated and Faure (1980) estimated that one person could measure between 500 and 1000 chicks per day. Large and

significant differences were established between active and inactive lines over an eight-generation study. The inactive or more fearful line had a higher resting level of plasma corticosterone at 2 weeks and about twice as high a level at 5 weeks when stressed, but differences were not found at 6 or 25 weeks of age (Faure, 1981b). Also, hens of the more fearful line consumed less feed for a few days when a different kind of feeder was used.

Tonic immobility

The phenomenon of induced tonic immobility (TI) has been known for centuries and its duration in chickens is generally interpreted as an indicator of fearfulness (Campo et al., 2012; Dávila et al., 2011; Gallup, 1974a; Jongren et al., 2010). That TI is influenced genetically was evident from large and moderate realized heritability estimates obtained in single-generation selection studies, respectively, by Gallup (1974b) with 21-day-old "Production Reds," by Campo and Carnicer (1993), and Anderson and Jones (2012) with White Leghorn hens. Also, Craig and Muir (1989) obtained a moderately large heritability estimate on White Leghorn hens which had been kept in three-bird cages for over 20 weeks. That the housing environment can have a significant effect on the duration of TI has been shown by Jones and Faure (1981), Kujiyat et al. (1983), Campo et al. (2008), and Lay et al. (2011).

Escape and avoidance ("flighty") behavior

Consistent and significant differences in escape and avoidance behavior of hens from two genetic stocks of White Leghorns were found for each of three stimulus situations believed to cause fear (Craig et al., 1983). Human observers were involved in two of the tests and a mechanical device was the primary source of stimulation in the third. In addition to stock differences, significant sire family effects, indicative of genetic variation within strains, were also found. Within genetic stocks, negative phenotypic correlations were detected between criteria used as indicators of fearfulness and feather loss; cages with more fearful hens lost more feathers presumably because of abrasion with cages and trampling and scratching. Cages held from 10 to 14 birds each. In a later study, involving hens of a random-bred White Leghorn stock kept in three-hen cages, Craig and Muir (1989) obtained heritability estimates ranging from a nonsignificant value of 0.08 to a significant 0.34.

Potential problems with selection on behavioral traits

Wegner (1990) suggests that welfare could be improved through adaptation by selecting against frustration, restlessness and stereotyped pacing before laying, and a greater tendency to sit during the prelaying period. However, direct selection on either behavior or physiological objectives should be

viewed with caution. The intended results may not be as expected. For example, Webster and Hurnik (1991) showed that traits associated with nonaggression, such as sitting and resting, were negatively correlated with productivity. Furthermore, the link between behavior and stress is misinterpreted. For example, Duncan and Filshie (1979) showed that a flighty strain of birds that exhibited avoidance and panic behavior following stimulation returned to a normal heart-beat sooner than a line of more docile birds, implying that docile birds may be too frightened to move. Therefore is flightiness good or bad for well-being?

An example of problems that can occur if selection is directed at the physiological responses to stress was provided by Gross and Siegel (1985). They were successful in selecting lines of birds for high and low plasma corticosterone in response to social strife. Further testing (Siegel, 1993) showed that the birds did not differ in their corticosterone response to a nonsocial stressor. Siegel concluded that genetic selection altered the perception of the animal to stress rather than involving the GAS directly. Even worse, Siegel (1993) noted that the low line in a low-strife environment was more susceptible to infections from endemic bacteria and external parasites while the high line in a high-stress environment was more susceptible to viral infections. Thus direct selection for low or high immune response would compromise the birds' welfare as measured by longevity. Also, correlated responses on productivity cannot be ignored. Birds with a high genetic potential for immunorespossiveness must divert energy from growth and reproduction to the immune system.

Selection involving production traits

Antagonisms

There is a natural antagonism between some undesirable behaviors and egg production. Research has shown that selection to improve productivity based on individual bird productivity was associated with increased aggressiveness (Bhagwat and Craig, 1977, 1978; Craig et al., 1975; James and Foenander, 1961; Lowry and Abplanalp, 1972; Lee and Craig, 1981a; Tindell and Craig, 1959). From the reverse perspective, Craig (1994) has shown that selection for social dominance will reduce performance when hens are housed in a large group, but when housed singly, performance is increased. Bhagwat and Craig (1978) found that social dominance increased in response to selection for egg mass. Alternatively, direct selection for social dominance has been shown to reduce performance when hens are housed in groups (Craig, 1994). Craig et al., 1965 and Craig and Toth (1969) showed that hens of White Leghorn lines selected for high social dominance had lower rates of egg production and higher mortality than did hens of the same line selected for low social dominance. In addition, Craig (1970) showed that the high-social-dominance line withstood crowing less well than the low-social-dominance

line. However, in single bird cages egg production of the high line was superior to that of the low. Biswas and Craig (1970) also showed that the high-strain hens had much lower production than the low line in floor pens or multiple-bird cages, but were equally productive in single-bird cage. Craig et al. (1975) demonstrated that stocks selected for part record egg production, based on individual performance, exhibited more social dominance and aggressiveness.

Other associations that are relevant include the finding that a stock selected for increased productivity had greater feather loss than its unselected control when kept in three-bird cages (Lee and Craig, 1981b). Also, Choudary et al. (1972) compared four commercial lines of poultry and found that the line which had the highest day rate of lay had the lowest hen-housed rate of lay due to high mortality. Results from Lowry and Abplanalp (1970, 1972) showed that strains selected under floor-flock conditions became socially dominant to both those selected in single-bird cages and unselected controls. Craig and Lee (1989) detected a strong genotype by beak-treatment interaction for egg mass per hen housed among three commercial lines. The genotype that produced the greatest egg mass with beak treatment produced the least with intact beaks. The re-ranking was shown to be due to mortality from beak-inflicted injuries.

Results from Emsley et al. (1977) suggest that flightiness is associated with higher productivity. They estimated genetic correlations between egg production and flightiness score which showed that greater excitability was mildly associated with higher rates of lay. Kashyap et al. (1981), attempting to break the correlation between production and flightiness, selected layers using a selection index for aggregate economic gain based on genetic parameters estimated by Emsley et al. (1977), which gave positive weight to egg number and negative weight to excitability or flightiness. Nevertheless, their results showed a positive response in excitability. Bennett et al. (1981) reanalyzed those with retrospective indices and showed that genetic changes in excitability were actually greater than what would have been predicted by theory.

The environment of selection

Biswas and Craig (1970) compared White Leghorn hens of lines selected for high- and low-social-dominance strains in single-hen cages and floor pens and in crowded and uncrowded conditions in both cages and floor pens. Significant interactions between genetic stock and environment were present with reversals in ranking between environments. In the first study, high social dominance hens matured earlier and laid more eggs per hen housed when kept in single-hen cages but were later in maturity and laid fewer eggs when kept in within-strain social groups in floor pens. In the second study, changes in rank of performance were also found for age at first egg and hen-housed egg production when the strains were compared in cage and floor pen environments.

High-strain hens were lower in performance than low-strain hens when compared in intra-strain groups in floor pens or in multiple-hen cages but were more or equally productive when kept in individual cages.

A significant genotype by cage-environment (single vs multiple) interaction for days' survival was observed in a random-bred population of White Leghorns (Muir, 1985). However, such an interaction can be caused by a change in variance or a change in ranking. Muir et al. (1992) showed that the interaction was mainly due to reranking of genotypes. Thus, the bird which does the best in one environment is much poorer in the other and vice versa.

Although there was no significant interaction for eggs per hen housed, Muir (1985) showed that this result was due to a quadratic relationship of surviving group size with rate of lay, whereby cages with seven surviving birds had a higher rate of lay than those with eight or nine. Estimation of genetic correlations assumes a linear relationship between genotype and phenotype. Therefore, to improve overall eggs per hen housed it is not only necessary to measure production in multiple-bird cages but to select separately on rate of lay and days' survival.

Therefore the problem of adaptation is a joint one: the environment of selection and the traits of selection. The usual solution to eliminate, or guard against, genotype by environment interactions in production animals, is to select the animal in the environment in which it is to perform, that is, to make the basis of selection performance in multiple-bird cages rather than in single-bird cages. However, that solution will not work in this case and could actually make the situation worse. Selecting the highest performing individual in a group environment will select for the more dominant birds.

>From a genetics perspective, the environment is not constant but is constantly changing because the environment is defined not only as that of a group but also of the individuals within that group. Individual genotypes in groups are constantly changing as selection progresses, that is, the associate effects of other genotypes in the population are involved. Thus, theory predicts that selection based on the individual will be antagonistic to group performance.

Although the studies cited indicated major effects of agonistic behavior on performance in egg-production stocks, most breeders select their elite stocks on the basis of records obtained in single-hen cages (Hunton, 1990). The genotype by housing environment interactions found in these studies emphasize the risk taken by breeders who select for egg production on the basis of hens' performance in single-bird cages for stocks to be housed commercially in social groupings.

Multilevel selection

Siegel (1989) considered adaptability to be an individual's ability to adapt to its environment. He concluded that individuals that adapt have a higher

probability of contributing genes to subsequent generations than those that do not. This concept emphasizes the individual. What if an individual adapts to its environment by eating its cagemates? Survival of the individual is maximized, but what of that of the group?

There are numerous ways that performance of one individual can influence that of another. Accommodation for such interactions presents an insurmountable dilemma from the point of view of classical (noninteraction) quantitative genetic methodology. Griffing (1967) recognized that with competition, the usual gene model for a given genotype must be extended to include not only the direct effects of its own genes, but also the associate contributions from other genotypes in the group. The problem is to optimize production of a given genotype in a competitive environment. As a consequence of interacting genotypes, the same genotype can have different expressions in populations having different population structures.

Griffing (1967, 1977) extended selection theory to take into consideration interactions of genotypes. The conceptual biological model was extended to define the group and the usual model was extended to include not only direct effects of its own genes, but also associate contributions from other genotypes in the group. It is also of special note that Griffing (1967) shows that selection only on associate components cannot guarantee a positive response to selection, that is, selection for reduced aggression will not ensure that production will increase.

The modern general classification of methods that use group selection or some combination of group and individual selection is now termed multilevel selection (Bijma et al., 2007a,b). Multilevel selection is the direct analogy of the classical index selection results based on the noninteraction model. Let Y_{kl} be the phenotype of the lth individual in the kth group (or family), then, the between and within group deviations can be combined in an index, $I_{kl} = B_1\tau k + B_2\gamma(k)_l$, where $\tau_k = \left(\overline{Y}_{k.} - \overline{Y}.\right)$ and $\gamma_{(k)l} = \left(Y_{kl} - \overline{y}_{k.}\right)$ are respectively the between- and within-group deviations, and B_1, B_2 are weights. Classical individual selection occurs when $B_1 = 1$ and $B_2 = 1$, between group selection occurs when when $B_1 = 1$ and $B_2 = 0$, and multilevel selection results when $B_1 \neq 0$ and $B_2 \neq 0$ (Muir et al., 2013).

Group selection experiments

There is limited, but ample, experimental evidence to support Griffing's (1967) theory. The first experiment was that of Goodnight (1985) who showed that leaf area of *Arabidopsis thaliana* would respond to group but not individual selection. The first experiment to use group selection with chickens was unsuccessful (Craig et al., 1982). Craig (1994) reflected that the reason for lack of response was because he had not provided an environment in which the hidden genetic variability could be expressed, that is, the beak trimmed hens, density was low, and only a part record was used.

The first successful experiment with group selection in poultry was initiated in 1981 at Purdue University. After four generations of group selection based on half-sister families housed initially in groups of nine, and later in groups of 12, performance was compared between the selected and control lines in six-hen cages with 387 cm^2 floor space per bird from 16 to 40 and 16 to 36 weeks of age, respectively, by Kuo et al. (1991) and Craig and Muir (1991). In the second study a stock derived from a competitive commercial stock by two generations of relaxed selection was included also. In both experiments, hens had their beaks left intact or beaks were trimmed to two different lengths.

Results of the first study showed highly significant beak-treatment by genetic stock by age interactions for hen-housed rate of lay and daily egg mass; as mortality from cannibalism increased dramatically with age in the control, but not in the selected stock, differences between the selected and control became greater. Somewhat similar results were obtained in the second study; with intact beaks, the selected line had significantly higher egg production, egg mass, and survival than its control. However, with two-thirds of the beak removed, differences in egg production, egg mass, and survival were no longer as evident. Differences in production were presumably due to stress induced by birds with intact beaks. In comparisons between the selected and the commercially derived stock, Craig and Muir (1991) found that egg production was at about the same level with all three levels of beak trimming. However, the selected stock had significantly better survival when beaks were left intact. Craig and Muir (1991) hypothesized that kin selection favored cooperative or at least tolerant behavior as suggested by Crow and Kimura (1970) and concluded that selection on family means when families are reared as family groups provides a method of improving traits in which behavioral interactions influence overall well-being and productivity.

Muir (1996) reported that after six generations, in comparison to the unselected control, annual percent mortality of the selected line in multiple-bird cages decreased from 68% in the initial generation to 8.8% in the sixth generation. Percent mortality in the sixth generation of the selected line in multiple-bird cages was similar to that of the nonselected control in single-bird cages (9.1%). Annual days' survival improved from 169 to 348 days and rate of lay improved from 52% to 68%. Annual egg mass improved from 5.1 to 14.4 kg per bird. The dramatic improvement in livability demonstrates that adaptability and well-being of these birds were improved by group selection. The similar survival of the selected line in multiple-bird cages and the control in single-bird cages suggests that beak-trimming of the selected line would not further reduce mortalities which implies that group selection can eliminate the need to beak-trim. An independent study by Craig and Muir (1993), involving different foundation stock, adds confirmatory evidence; kin-selection for days survival of intact-beak hens was dramatically increased relative to the unselected control over a two-generation

study. In the Muir (1996) study, corresponding improvements in rate of lay and egg mass demonstrated that such changes can also be profitable.

Craig and Muir (1996) compared the selected and control lines to a commercial line in generation 7. Birds were not beak-trimmed and lights during the laying period were set to high intensity. Birds that died were replaced with extra birds of the same line. In single-bird cages performance, as measured by eggs per hen housed, the commercial line was superior to that of the selected line but in 12-bird cages performances were reversed. The difference in ranking was due to both an improved rate of lay and viability. In the same study, Craig and Muir (1996) observed that feather scores did not differ in single-bird cages among genetic stocks. However, in 12-bird cages, the selected line had significantly better feather score than the other lines.

The lines, described by Craig and Muir (1996), were subjected to the stress of housing at about 17 weeks, to cold stress at 36 weeks, and to heat stress at 47 weeks of age, with results as reported by Hester et al. (1996a, 1996b). Blood physiology and egg production were monitored before, during, and after each of these periods. Packed cell volume immediately after housing indicated that the selected line adapted to the new watering system more quickly than the other lines. During cold stress the commercial and control lines showed an increase in heterophil to lymphocyte ratio in 12-bird cages while the selected line did not. Egg production before, during, and after stress indicated that the selected line withstood social, handling, and environmental stress better than the control and in some case the selected line. Similar observations with heat stress showed that the selected line withstood heat stress better as indicated by a lower mortality than the control or commercial lines. Egg production before, during, and after heat stress indicated that the selected line withstood social, handling, and environmental stress better than the control line and in some cases the commercial. Cheng et al. (2001a, b) studied the same line compared with the revised selected line (line with low production and high mortality) housed in single-bird cages. At 21 weeks, the selected line had a higher CD4 to CD8 lymphocyte ratio and lower concentrations of dopamine and epinephrine and lower ratios of epinephrine to norepinephrine and heterophil to lymphocyte ratios. The bidirectional selection has resulted in the change in the immunity and the expression of the neuroendocrine systems. In social stress studies using the same lines plus a commercial line, DeKalb XL, Cheng et al. (2002, 2003) and Cheng and Fahey (2009) reported that the selected line has higher resistance to various social stressors than the other two lines. These changes magnify the differences in productivity and survivability observed in the lines under basal and challenged conditions.

Conclusions from group-selection studies

General conclusions were summarized by Muir (2003) and Muir and Cheng (2004). Selection to reduce the major economic and welfare problems of

feather pecking and cannibalism among laying hens may be successfully achieved by either direct selection against pecking injuries or by means of correlated responses associated with group (kin) selection for adaptation to multiple-hen cages as indicated by mean hen-day rate of lay and survival.

Multilevel selection experiments

Although (Griffing, 1977) had developed equations to determine optimal weights for between- and within-group deviations, those methods were only published in a conference proceedings and were largely unknown. Further, the results were academic at the time because methods to estimate genetic parameters associated with the model did not exist as Henderson's (Henderson, 1975, 1984; Henderson and Quaas, 1976) mixed model methods were just being developed and was limited to models with one random effect.

In order to estimate genetic parameters associated with the model, Muir and colleagues (Muir, 2005; Muir and Schinckel, 2002) recast Griffing's model in terms of a mixed model methodology, with two random effects, one for direct effect of the allele on the phenotype and another for effects on associates. Griffing termed the social or competitive effects, as "associative effects," which are now commonly referred to as indirect genetic effects (IGEs) (Agrawal et al., 2001; Bijma, 2010a,b; Bijma and Wade, 2008; McGlothlin and Brodie, 2009; Wade et al., 2010). An important result of the mixed model approach of Muir (2005) and later (Bijma et al., 2007a) is that genetic variances and covariances associated with direct $\left(\sigma_D^2\right)$ indirect $\left(\sigma_A^2\right)$, and their covariance (σDA) could be estimated by use of REML (Patterson and Thompson, 1971). These genetic parameters, in combination with weights for the between- and within-group deviations, unified the concepts of multilevel (kin and nonkin), group, and within group selection (Bijma et al., 2007a, b; Griffing, 1977). Bijma et al. (2007a,b) used the term g to define the strength of multilevel selection, ranging from 0 to 1 corresponding respectively to within and between group selections.

As a result of the two-component model of Muir (2005), the direct and IGEs can be directly estimated for each individual using best linear unbiased prediction (BLUP) (Henderson, 1975, 1984; Henderson and Quaas, 1976). As a result, any combination of weights could be placed on those effects resulting in optimal selection (Bijma et al., 2007b). Muir and colleagues (Muir, 2005; Muir and Schinckel, 2002) used this approach, in comparison with individual selection, to select for increased 6-week weight in Japanese quail housed in multiple random groups of size 16. We used a base index in which the IGEs were summed over all $(n-1)$ interacting genotypes in a group of size n, plus the direct effect known as the total breeding value [TBV, (Bijma et al., 2007a, b)]. We demonstrated that selection for TBV was effective in increasing 6 week weight while reducing mortality. In

contrast, individual selection did not increase 6 wk wt. while having the negative effect of increasing the rate of mortality.

An interesting consequence of defining the index as the trait of selection, in terms of the TBV, is that the total heritable variance can exceed the observed phenotypic variance (Bijma et al., 2007a) indicating that the response to selection can be greater than the selection differential, and explains the results we observed with poultry where the realized heritability in the initial generations were >1. Basically the response to selection includes the heritable social environment, not just the direct effects

Muir and Schinckel (2012) examined multilevel selection for quail housed in kin groups for 43 days weight and survival in Japanese quail. We showed that multilevel selection occurs whenever BLUP selection is applied if quail are housed in groups because BLUP weights all information from relatives, in addition to that of the individual. If families are housed in kin groups, then BLUP selection has elements of both kin and group selection. We showed that multilevel selection in kin groups was effective in reducing detrimental social interactions, and reduced mortality, which contributed to improved weight gain. Simple multilevel selection using standard animal model BLUP was easy to implement, only requiring that animals be housed in kin groups.

General conclusions

The wild progenitors of domesticated chickens possessed a number of traits, including absence of highly specific environmental requirements that pre-adapted them to domestication. They continue to show adaptability and have responded to man's selection as seen by wide diversity among breeds and types. Nevertheless, because of large and rapid changes in housing systems and husbandry practices during the last 50 years, questions arise as to whether their welfare is compromised and whether genetic adaptation has been adequate. Criteria used in assessment of chickens' well-being include overt indicators (bodily injury, morbidity, weight loss, nonmolt feather loss, death, and mean days survival of groups), physiological and immune responses, productivity, and behavior. With the exception of overt indicators, difficulties of interpretation of the various criteria lead to the conclusion that, so far as possible, multiple indicators should be used. Nevertheless, because behavior is the means by which animals attempt to cope with their environments, behavioral observations can yield valuable clues as to what is causing impaired welfare, and it is clear that behavior-related problems can themselves have major adverse consequences.

Most behavioral differences found among domesticated stocks of chickens can be shown to be determined genetically and are under polygenic influence. However, it is often difficult to know whether those differences arose because of foundation population differences, random genetic drift, adaptation to local environments, or different goals under artificial selection.

The tendency to assign behavioral differences to breeds needs to be moderated when only a single strain within a breed has been used for characterization. Long-term selection studies for behavioral or economic traits that succeed have shown that profound correlated responses are likely to accompany the primary responses.

Although selection to improve behaviors associated with well-being has been effective in most experimental studies, commercial breeders are under economic constraints that may prevent their ready adoption of programs to address those problems. Behaviors that are easily identified (such as broodiness) or that leave traces (such as feed consumed) are more amenable to selection than those that must be measured by time-consuming observations (e.g., prelay pacing, fear-related and aggressive behavior). It has also been noted that direct selection could be disappointing if such selection reduced efficiency of production. It has been shown that kin selection was effective in reducing a major social-behavior problem when the consequences were readily evident. Thus cannibalistic behavior by quail was reduced by selecting individuals on the basis of weight when kin were caged together. This method is easy to implement and does not require behavior to be directly measured. The impacts of behavior are evident in performance traits. An alternative approach, also utilizing kin selection for improvement of well-being of birds in multiple-hen cages, was based on the hypothesis that when productivity is high, behavioral stresses must be reduced or absent. Thus increased adaptation of layers to multiple-hen cages was attained by selecting families that had high egg production and survival even when kept together under conditions that would stress less-well-adapted stocks (hens with intact beaks in high-density, larger-group-size cages). That an impressive response was obtained within a few generations indicates that this approach deserves further attention commercial conditions and that eventually management practices such as peak trimming will no longer be necessary.

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Chapter 13

Genetics and animal welfare

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ABSTRACT
Overselection for production traits has caused animal welfare problems such as feather pecking in hens, tail biting in pigs, and overly aggressive animals. In dogs, overselection for appearance traits has caused neurological problems such as deafness. Both feather pecking and tail biting may be displaced foraging behaviors, because these behaviors are reduced by providing foraging materials such as straw. Another problem is hunger in broiler breeder hens and breeding sows. Animals that have been selected for rapid growth are also selected for a huge appetite. If breeder animals eat to satiation, they will become obese and have health problems. High-roughage feeds may improve welfare. Researchers suggest that new genetic breeding tools could be used to select against harmful behaviors and still have a productive animal. Breeders must avoid creating animals that will have poor welfare even when they are housed in the best environment.

Introduction

The productivity of domestic livestock and poultry has almost tripled in the last 100 years through the use of both improved feeding methods and genetic selection. Freeman and Lindberg (1993) state that enhancements in genetic selection in the dairy industry have contributed more to increased milk production than improvements in management. The National Milk Producers Federation (1996) reports that during a 30-year period, milk production in Holstein cows more than doubled. Since the first edition of this book was published in 1998, milk production has had an additional 14% increase from 1999 until 2009 (USDA/NASS, 2010). Since the 2014 edition, there has been an additional 11% increase in milk production (USDA National Agricultural Statistics Services, 2019). Data from the United Kingdom show that from 1995 until 2012, milk production increased by approximately 30%.

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Similar gains have been made in poultry (Gordy, 1974; Maudlin, 1995). Due to genetic selection, the ability of a chicken to gain weight has increased phenomenally. In 1923 it took 16 weeks to produce a broiler chicken. Today, only 47−48 days are required to grow a boiler chicken to 2.98 kg (6.41 lbs) (National Chicken Council, 2021). In 1990 a chicken fed for the same number of days only reached 1.98 kg (4.37 lbs). This is a 30% increase in the growth rate.

Adverse effects on bird welfare are often a consequence of selective breeding for high-production traits. The ancestors of the modern-day chicken laid about 25 eggs a year. Modern laying hens produced 310 eggs a year in 2010 and 325 eggs a year in 2015 (Preisinger, 2018). After about a year they are considered "spent." In the meat sector, broiler chickens can convert 3 kg of food into 2 kg of meat (Robbins and Phillips, 2011). To put this growth rate into perspective, Boersma (2001) calculates; "If you grew as fast as a chicken, you'd weigh 349 pounds at age 2." Selection for rapid growth rate has resulted in decreased heart and lung size relative to the rest of the body (Decuypere et al., 2007), and in skeletal defects that affect walking ability (Corr et al., 2003; Knowles et al., 2008). Although many breeding companies now increasingly incorporate health and welfare goals alongside economic goals, from a welfare point of view, the growth rate is still seen as a problem (Cooper and Wrathall, 2010; Rayner et al., 2020). The percentage of deaths in heavy 2.998 kg (6.41 lb) broiler chickens is 5% (National Chicken Council, 2021).

For years, both authors have been concerned that in the future, the most serious animal welfare problems may be caused by overselection for production traits such as rapid growth, leanness, and high milk yield. Unfortunately, many of these concerns have materialized. Many scientists are worried about indiscriminate selection for production traits that seriously compromise animal welfare. Today, many Holstein dairy cows last for only 2 years of milking. Many producers now realize that selecting for an even increasing amount of milk production may be a mistake. Crossbred dairy cows are now more popular. There will always be tradeoffs. A highly robust animal that can live outside may be less productive (Dixon, 2020; Tamou et al., 2018). There may be a conflict between welfare and sustainability. Highly productive animals require less feed and the same amount of meat, milk, or eggs can be produced with fewer animals. Breeders need to start thinking about optimum and not maximum production.

Turner (2011) warns that selection for economically important traits may be a factor in the expression of behaviors detrimental to welfare. Some examples are aggression in pigs and feather pecking in laying hens. The use of genomic information has the potential to help breeders select against harmful behavior, but if it is used indiscriminately, it could accelerate the selection of harmful behaviors. Prunier et al. (2010), Jensen et al. (2008), Rodenburg and Turner (2012), Brito et al. (2020), and Colditz and Hine (2016) all state that new breeding tools could be used to improve animal welfare by selecting against harmful behaviors.

Several long-term selection studies using a variety of small mammals have clearly shown that overselection for a single trait may have adverse or unexpected effects on other traits (Belyaev, 1979; Dobzhansky, 1970; Lerner, 1954; Wright, 1978). Belyaev (1979) and Belyaev and Borodin (1982) demonstrated the effects of overselection for a single trait in long-term selection experiments in foxes. Selecting foxes for a single behavioral trait (tameness) caused unexpected changes in coat color, breeding cycles, hormonal profiles, and subsequent changes in body traits. In 2005 Grandin and Johnson (2005) wrote about extremely aggressive broiler breeder roosters that killed hens. Producers had to cut off his front toes to reduce losses. In 2018 and 2021 the first author visited two broiler breeder farms that had roosters from the same breeder. The aggressive rooster problem had been solved and producers stopped cutting off his toes (Fig. 13.1A and B). Unfortunately, the breeding company never published a scientific article about how the welfare of broiler breeders was improved by selecting for less aggressive roosters.

In domestic pigs, selection for meat production traits has resulted in animals with the lowered reproductive ability (Dickerson, 1973). To compensate, modern pork producers often use maternal sow lines selected for high reproduction ability, bred to sire lines selected for high meat production. The resulting offspring are terminal cross pigs fattened for market. Since the publication of the second edition in 2014, highly prolific sows are now being used. The sow sometimes has more piglets than she can nurse. Ward et al. (2020) ask an ethical question, "How many pigs died to achieve the numbers weaned?" In the 1990s, most commercial market pigs were crossbred. Today, the use of hybrid sire and dam genetic lines is still a standard practice. Genetic selection of highly inbred lines with extremely fast growth rates is associated with biochemical disorders. Some of the metabolic diseases



FIGURE 13.1 Broiler breeder roosters from a major breeding company have been selected to be less aggressive. (A) Typical broiler breeder male. (B) His three front claws and his toes no longer have to be removed to prevent injury to the hens. Fifteen years ago, the routine practice was to cut all three toes off at the first joint.

associated with genetic selection are Mulberry heart disease (Shin et al., 2011), porcine stress syndrome (Leman et al., 1992), and osteochondrosis, a disease of bone and cartilage growth (Stern et al., 1995). Of particular interest, fast-growing pigs display patterns of genetically determined pathologic alterations similar to those observed in human populations afflicted by obesity (Cummings and Schwartz, 2003).

With the exception of a short section on biotechnology at the end of this chapter, all welfare problems discussed here were created with conventional breeding methods. The animals are not GMOs (genetically modified organisms) or products of biotechnology.

Aggression in pigs

Practical experience shows that certain genetic lines of sows produce efficiently in stalls but have high levels of fighting injuries when housed in groups. Pig production companies that have successfully moved from gestation stalls to group housing have done so by changing genetics and selecting for less aggressive sows. When sows were kept in individual stalls, there was no informal selection by producers to eliminate the most aggressive animals. Grandin (2018) observed big differences in the behavior of Dutch and German intact market weight boars at the slaughter plant. The German boars quietly laid down after arrival. The Dutch boars constantly fought and mounted each other. Another interesting observation is that many of the German boars had only one testicle. From a welfare perspective, the German intact males had better welfare. Larzul (2021) reported that selection against both boar taint and aggression may have detrimental effects on reproduction. There is always a tradeoff.

Research clearly shows that aggression in sows is heritable (Brajon et al., 2020; Chu et al., 2017; d'Eath et al., 2009a). Chapter 9 of this book, by Lotta Rydhmer, covers the genetic factors that affect sow aggression. Aggression interacts with other behavioral traits in odd ways. Purebred Yorkshires and Yorkshire × Landrace pigs that stood still on a scale during weighing were less aggressive and took longer to move in and out of the scale (d'Eath et al., 2009b). Fortunately, selecting less aggressive sows does not affect maternal ability (Lovendahl et al., 2006). On-farm observations indicate that pigs bred for lower aggression must never be mixed with more aggressive pigs. Low-aggression pigs suffer many attacks and may become severely injured.

Tail biting in pigs

Breuer et al. (2004) found that tail biting was heritable in Landrace pigs and not in Large Whites. Breuer et al. (2004) found that genetic selection for lean growth and low back fat is associated with tail biting in Landrace pigs.

This agrees with the first author's observations of thousands of market pigs at many US slaughter plants. A much higher percentage of bitten tails is seen in certain genetic lines of pigs. Pigs from these lines are more excitable and chew harder on a person's boots compared to other genetic lines. Taylor et al. (2010) has an interesting hypothesis and suggests three types of tail biting, each with different motivations. The differences are referred to as; "sudden," "forceful," and "obsessive" (Taylor et al., 2010). Prunier et al. (2019) has identified two types of tail biting that may have different motivations. There is aggressive tail biting and nonaggressive tail biting that is motivated by the urge to chew and explore. Tail-biting pigs spend more time manipulating enrichment devices (Zonderland et al., 2011), and victims of tail biting are more aggressive than pigs that bite tails. Misdirected foraging is very different than aggression (Zonderland et al., 2011). On a novel object test, tail biters had lower anxiety compared to victims (Zupan et al., 2012). Sinisalo et al. (2012) report breed differences in tail biting and found that when Yorkshire and Landrace pigs are mixed, the Yorkshires are more likely to be victims of tail biting. When the first author visited Japan, she observed confinement-reared pigs that did not have to have their tails docked to prevent tail biting (Fig. 13.1). Van der Weerd et al. (2008) found that the use of straw and chewable enrichment devices may help to prevent severe tail biting. In this study, three enrichment procedures were used; straw, a rootable feed dispenser, and a feed dispenser providing flavorful water and chewable rods that could be manipulated. Results show that a full bed of straw was the most successful way of occupying the pigs. Pigs could manipulate the straw even while lying down. In modern swine confinement systems, straw bedding causes serious problems with waste management systems. Producers want enrichment devices that do not clog up their systems. Chou et al. (2020) found that a rubber toy for nursery pigs and a spruce wall-mounted wooden post reduced tail biting. Feeding a higher fiber diet made tail biting worse (Chou et al., 2020). Buijs and Munns (2019) researched nonstraw enrichments. They found that ropes and fresh wood hessian sacks reduced tail biting. The objects must be changed frequently (Buijs and Munns, 2019). Age may also influence aggression and tail biting. Li et al. (2012) suggest that young sows are vulnerable in group-gestation systems when mixed with mature sows. Sorting sows by the number of times they have given birth improves welfare and performance by reducing severe injuries and fighting.

Savaging in sows

Sows killing their piglets is an example of an extreme welfare problem that may interact with environmental conditions. Sows that savage are more restless at farrowing. Ahlstrom et al. (2002) suggest that aberrant maternal behavior during farrowing or an individual's inability to cope with restrictive

environments may be contributing factors in savaging. Chen et al. (2008) compared behaviors such as grinding teeth, rearing, lying down with care, and prepartum nest-building behaviors. These behaviors were not a predictor of savaging. However, Chen et al. (2008) reported that sows that made more postured changes during farrowing were more likely to savage. This suggests that savaging is part of a more generalized behavioral pathology that includes increased excitability—not specifically piglet directed. Baxter et al. (2011) found that modifying the farrowing environment affected maternal behavior in sows, and suggest that it is essential to evaluate sows in the environment they will be housed in. This is especially important when sows from different genetic lines are being switched from indoor, intensive environments to outdoor environments. Sows attacking and killing their own newborn offspring is a serious welfare concern that requires further study.

Feather pecking in hens

In the egg-production sector, feather pecking is a serious welfare and economic problem. Recent studies have focused on behaviors of genetic lines of hens selected for either high or low feather pecking. Evidence suggests that feather pecking in laying hens may be misdirected foraging behavior (deHaas and van der Eijk, 2018). Rodenburg et al. (2010) investigated the underlying motivation of feather pecking in genetic lines of chickens bred for high and low feather pecking. Results suggest that fear may not be the primary motivator, as the two genetic lines had similar reactions to the novel object test and human-approach test (Rodenburg et al., 2010). A recent study done by Iffland et al. (2020a,b) indicates that in extremely high feather peckers, high fear levels were associated with extreme pecking. It has also been suggested that dietary factors could play a role in feather pecking, however, hens selected for high feather pecking ate more of a high-fiber diet (Kalmendal and Bessei, 2012). Lines selected for increased feather pecking showed increased motivation for locomotor activity (Kjaer, 2009). deHaas et al. (2010) found that a high feather-pecking line ate worms faster. This is further evidence that feather pecking is displaced foraging. High-producing animals require vast quantities of food. The first author speculates that selection for high egg production and increased appetite are related. Fisikowski et al. (2008) found that two subhaplotypes of the DRD4 gene are associated with feather pecking. Dopamine genes are associated with exploratory behavior in many species. This finding is additional evidence that foraging behavior and/or increased appetite is related to high feather pecking. Behavioral observations also show that hens perform gentle pecks during dust bathing and vigorous pecks when foraging. Pecking behavior directed toward other birds resembles pecking behavior during foraging (Dixon et al., 2009). In a subsequent study, the same research team showed that providing environmental enrichment opportunities for foraging is effective in reducing

feather pecking (Dixon et al., 2010). Kjaer and Jorgensen (2011) found that birds from a high-feather-pecking line had higher autonomic nervous system arousal during physical restraint compared to those from a low-feather-pecking line. Brunberg et al. (2011) suggest that feather pecking may serve as a model for human psychiatric disorders. These studies provide a gene list that may be useful in further research on the mechanisms behind feather pecking (Brunberg et al., 2011; Falker-Gieske et al., 2020). The latest research shows that feather pecking is influenced by many genes (Iffland et al., 2020a,b).

Effects of overselection in cattle and pigs

Since 1971, the first author has observed hundreds of thousands of animals at slaughter plants, farms, and feedlots in the United States, Canada, Europe, Australia, and New Zealand. In the early 1990s, the first author began to observe an increased number of highly excitable, nervous pigs and cattle. These animals were more difficult to handle and more likely to panic and become extremely agitated when subjected to sudden novel experiences. For example, a light tap on the rear causes squealing in pigs with an excitable temperament, but a tap on the rear has little effect on pigs with a calmer temperament. The appearance of highly excitable and difficult-to-handle animals appeared to coincide with genetic selection for both rapid growth and high, lean-meat yield (Grandin, 1994). These observations were made over 30 years ago but it is important to write about them so today's breeders do not repeat these mistakes. In cattle, the most reactive animals are primarily crossbreeds from the European continent. In the United States, these cattle became popular when producers started selecting lean beef (Grandin, 1994). Along with welfare problems and handler safety issues, highly excitable animals have an increase in meat-quality problems. Cattle that become highly agitated during handling are more likely to have tough meat and more dark cutters (Voisinet et al., 1997). Many studies now show an association between poorer meat quality and excitable temperaments in cattle (del Campo et al., 2010; Muchenje et al., 2009; Turner et al., 2011), and pigs (Barton-Gade, 1984; Sayre et al., 1964). Since the second edition of this book in 2014, an industry-wide acceptance of this problem has motivated producers to select intensively for calmer cattle (see Chapter 4 on cattle temperament testing). The results are calmer and easier to handle cattle with better meat quality (Ribeiro et al., 2012). However, increased selection for calm temperaments is swinging the pendulum the other way, and concerns have now shifted to problems associated with a loss of beneficial foraging and maternal traits in calm cattle.

In both the United States and Australia, cattle are grain-fed in large outdoor feedlot pens. During the 1970s, the first author never observed tongue rolling or other abnormal behaviors in grain-fed cattle. In a 1996 visit to

several large feedlots, she noticed an increase of "stereotypic" (abnormal) tongue rolling in fed Holsteins. Some Holstein steers obsessively lick fences, gates, or other surfaces in the feedlot pen. This behavior is expressed even though cattle were fed (ad lib.) grain and corn. Licking is so obsessive that Holstein steers learned to lick open gate latches. Beef-breed cattle in feedlots never engage in constant licking or tongue rolling.

Both authors speculate that continuously selecting for high milk production and increased appetite may explain the increase in licking and tongue rolling. A large amount of food is required for Holstein cows to produce such large amounts of milk, and the effects of increased appetite are also revealed in male offspring of these cows.

From a welfare standpoint, grain-fed Holstein steers are probably not compromised, but from a health standpoint, welfare problems already exist. The first author has observed that Holstein steers on a high brain ration bloat more often compared to beef breeds on similar rations. In addition to bloat, grain-fed Holsteins have more sudden death than beef cattle. Feed yard employees have observed healthy-appearing Holsteins suddenly fall over and die while standing at a feed trough. Holstein cattle have a higher percentage of animals with liver abscesses (Amachawadi and Nagaraja, 2015; Trotter, 2016).

Muscle growth and welfare

In the second edition of this book, we discussed the compromised welfare of poultry by genetic selection for rapid growth and high meat production. The growth of the skeleton and the internal organs cannot be kept up with the growth of muscle mass in broiler chickens selected to grow very quickly, resulting in lameness and leg deformities (Muir, Chapter 12). Today, a combination of selective breeding and changes in feeding practices has reduced leg problems. For example, a lower-energy feed is given to young chicks to slow down growth slightly and allow the leg bones to develop. However, broiler chickens continue to have reduced cardiopulmonary capacity in relation to their muscle mass, and cannot withstand much physical exertion (Broom, 1987, 1993; Julian, 1993; Julian et al., 1986; Gall et al., 2019). Heavy broilers have little stamina and lie down after walking a few meters. Gall et al. (2019) report that market-weight broiler chickens with wooden breasts were not able to right themselves if they were laying on their backs. Wooden breast is a muscle defect that occurs in some fast-growing broilers. They also have a reduced range of motion of their wings. Reproductive fertility has also been reduced by extensive selection for rapid growth—muscle growth in the breast can make mating difficult. Turkeys selected for large breasts are unable to breed naturally (Dinnington et al., 1990). Another example is Belgian Blue cattle. This breed is so heavily muscled that a high percentage of their calves must be delivered by cesarean section

(Broom, 1987, 1993). Not only do births of such calves require surgical intervention, but their tongue muscles are also sometimes too enlarged to suckle, leading to death (Lips et al., 2001; Uystepruyst et al., 2002). Fortunately, some Belgian Blue genetic lines are now available that have much easier calving. The genetics of the double muscle trait have been extensively studied. Selection for this trait is associated with the inactivation of the myostatin gene, coinciding with reduced organ mass (Fiems, 2012). A mutation causes the myostatin gene to become inactive, resulting in muscle hypertrophy, and coinciding with a susceptibility to respiratory disease, kidney stones, lameness, heat and nutritional stress, and dystocia. In addition, Belgian Blue cattle require a diet high in nutrients, due to a reduced feed intake capacity (Fiems, 2012). In 2012 the first author visited a farm in the United Kingdom where Belgian Blue sires were bred to Devon × Limousin cows enabling the production of heavily muscled calves that required no growth promotants. The huge Devon × Limousin cross cows required a high-nutrient diet that worked well in the extensive, lush pastures in the United Kingdom, but some nutrient-poor pastures in the United States would not be able to support these cattle. High lean-meat yield is desirable, but it is important to find a balance between good performance in double-muscled animals and acceptable welfare. In the United States, ranchers who graze cattle on pastures with sparse vegetation are switching to smaller beef cows.

Porcine stress syndrome and leg problems in pigs

In the past, some pork producers deliberately used boars positive for the porcine stress syndrome (PSS) gene. The crossbred offspring from these boars have a higher percentage of lean meat and larger loin eyes (Aalhus et al., 1991). PSS is inherited in a classical Mendelian manner. Pigs that are either homozygous negative or heterozygous with one PSS gene (carrier state) will not display the symptoms of PSS when they become excited. Furthermore, when a homozygous-positive PSS boar is bred to a sow that is free of the PSS gene, none of the offspring will be homozygous-positive for PSS. To maximize kilograms of lean meat, swine-breeding companies sell PSS homozygous-positive boars for breeding to PSS homozygous-negative sows. A welfare problem is created if a producer breeds PSS-heterozygous sows (carrier state) to PSS-homozygous boars. Unfortunately, this practice is quite common because a producer can breed terminal cross gilts from his own herd instead of buying new gilts that are homozygous-negative and completely free of the PSS gene.

The first author has observed that some of the lean hybrid pig lines developed in the 1990s often had five times as many death losses, compared to older genetic lines of pigs with more back fat. In the 1990s, truck drivers transporting pigs often reported one or two dead pigs on a truckload of hybrid market animals. These deaths were likely to occur even when pigs

were transported under good conditions. Older genetic lines often had no dead pigs on a truck. Many of the truckloads with high death losses contained pigs sired by PSS-positive boars. Murray and Johnson (1998) reported higher death losses in market pigs with the halothane PSS gene. In the 2000s, the use of PSS genetics declined and death losses are now lower. USDA figures show that dead pigs arriving at the slaughter plants dropped from 0.29% in 2000 to 0.18% in 2016.

Although the PSS problem had been reduced and the US Pork Industry has now almost eliminated PSS genetics, other problems associated with a demand for lean meat and larger loin eyes are occurring. Beta agonist feed additives (ractopamine) are now used to enhance the growth of lean muscle. High doses of ractopamine can cause weakness, downed pigs, hoof cracking, and increased aggression (Grandin, 2020; Marchant-Forde et al., 2003; Poletto et al., 2011; Peterson et al., 2015). Feeding the β-agonist ractopamine can suppress the expression of serotonergic genes in the brains of the pigs (Poletto et al., 2011). Problems corrected by eliminating PSS genetics may now be repeated by using drugs.

As the US industry was phasing out PSS genetics, new problems with poor leg conformation and lameness started to occur. Single-minded selection for large loin eye size, thin back fat, and rapid growth resulted in a neglect of selecting breeding stock with good leg conformation. In the early 2000s, the first author observed approximately 50% lame market-weight pigs in some herds. A recent Finnish study showed that certain genetic lines of pigs selected for feed efficiency had greater percentages of lame pigs (Meunier-Salaun et al., 2018).

High death loss in ultralean hybrids was a more serious problem in the United States than in Europe. Death losses in Europe were much lower for the same hybrid pig lines from the same commercial breeding company. The reason for this may be that European pigs are fed a limited grain ration and are grown more slowly. Slower growth results in older market-weight pigs that have a more mature skeleton. In the United States, market pigs are fed ad lib. grain and attain market weight at an earlier age. Another factor influencing increased death losses and nonambulatory pigs in the United States is higher slaughter weights compared to pigs in Europe. The first author has observed that heavier animals are more likely to collapse during physical exertion.

Problems caused by genetic selection for appetite

Animals genetically selected for rapid weight gain or production of large quantities of milk, eggs, or meat require a tremendous appetite and food intake to produce large amounts of these products. Selection for rapid muscle growth correlates highly with selection for increased appetite. Research on chickens shows that birds selected for egg production stop eating when their

metabolic needs are met, but broiler chickens selected for meat production do not stop eating until their gut is completely full (Nir et al., 1978).

From an appetite standpoint, the welfare of broiler chickens and market pigs being fattened for slaughter is good. These animals are allowed to eat until satiated. However, a welfare problem related to increased appetite occurs in breeder hens and sows of the fast-growing offspring. Breeders have to be maintained on a calorie-restricted diet (Close, 1996). For example, broiler breeders on a restricted diet produce more eggs (Robinson et al., 1991), but if allowed to eat until satiated, they develop reproductive problems (Yu et al., 1992a). Furthermore, if sows are fed all they can eat, they become over-fat, which can result in leg problems and difficulties farrowing. Problems occur because breeder animals' appetites far exceed their basic metabolic needs.

Broiler breeders used for meat production are fed 60%−80% less than they would eat if fed ad lib. to prevent them from becoming overweight (Karunajeewa, 1987; Hocking, 1993; Hocking et al., 1993; Yu et al., 1992a, b; Zuidhoff et al., 1995). When hens go into egg production to produce broiler chicks, they are restricted to 25%−50% of what they would eat if they ate until satisfied. Feed restriction in sows is slightly less extreme compared to broiler chickens. Gestating sows are fed approximately 60% of their ad lib. intake of a standard grain concentrate diet (Lawrence et al., 1989). A sow nursing piglet is allowed to eat all she wants, but during gestation, she is kept on a calorie-restricted diet to prevent her from becoming too fat.

A review of a number of studies shows that feed restriction in breeding sows and chickens results in many abnormal behaviors, such as stereotypies (Lawrence and Terlouw, 1993). Appleby and Lawrence (1987) and Tolkomp et al. (2005) found that stereotypies developed in sows only when their feed intake was restricted. In a subsequent experiment, the same research team concluded that the amount of feed sows are fed on commercial farms is not high enough to satisfy their motivation for feeding (Lawrence et al., 1989). They further concluded that hunger resulting from a restricted diet may be a major cause of stress in confined housing systems. Bergeron and Gonyou (1997) found that stereotypies developed when the diet did not have sufficient energy to prevent hunger. Similar results have been found in other animals. Savory et al. (1992) found that feed restriction in broiler breeder hens also results in stereotypies. It is becoming increasingly clear that feed restriction in animals selected for increased appetite is a serious welfare concern.

Importance of roughage feeds

It is a common practice in the poultry and pork industries to feed highly concentrated diets consisting of mostly grain to breeding sows and broiler breeders. This does not compromise the welfare of market animals that are fed ad lib., but it may increase welfare problems in calorie-restricted breeding

animals. Studies in poultry, pigs, and cattle show that the incidence of abnormal behavior can be reduced by providing roughage in the diet (Lawrence et al., 1989; Redbo and Norblad, 1997; Robert et al., 1993; Zuidhoff et al., 1995). Roughage helps to reduce stereotypies and other abnormal behavior because diets high in roughage can be restricted in calories and fill the animal's gut. Diets with added roughage also take more time to eat and satisfy the animal's motivation for mouth activities. However, artificially increasing food intake time by placing hanging chains in the feed trough failed to reduce the development of stereotypies (Bergeron and Gonyou, 1997). The wild ancestors of chicken and pigs spent many hours pecking and rooting to obtain their food. Stolba and Wood-Gush (1989) report that domestic swine reared in woods and grasslands display most of the same behaviors as their ancestor, the European wild boar. Behaviors used for obtaining food, such as grazing and rooting, occupied large portions of each day. Even though the adult domestic sows used in this study came out of an intensive housing system, they quickly reverted to ancestral foraging patterns.

It is important to provide sows with roughage feed that provides both gut fill and mouth activities. Feeding roughage such as straw, sugar beet pulp, or oat hulls can improve the welfare of breeding animals kept on a calorie-restricted diet (Close et al., 1985). Zuidhoff et al. (1995) found that feeding concentrates diluted with 15% oat hulls increased the time required to consume the feed and reduced stress. Stress was measured with a heterophil/lymphocyte test. Diets high in fiber (lignocellulose) were effective in reducing feeding motivation (Dejong and Guemene, 2011; Souza de Silva et al., 2012). Chopped straw was added to the ration and hunger was measured by counting how many times boars would press a panel to obtain feed rewards. Whole straw provided to pigs is more effective for reducing abnormal behavior than chopped straw. Stereotypies in sows housed in individual stalls can be prevented by feeding small amounts of straw (Fraser, 1975). Compared to baled hay, pelleting, or wafering, roughage feeds decrease bulking by about 75% (Haenlein et al., 1966). Willard et al. (1977) found that horses fed pellet diets spent more time chewing wood and eating manure compared to horses fed hay.

Some sectors of the industry have been reluctant to feed roughage because roughages are too bulky to move through some types of automated feeding systems. Roughages are also more expensive to transport and increase the load on manure-handling systems. Management issues aside, adding roughage to the diet improves productivity. Broiler breeder hens fed a bulky diet of 15% oat hulls had higher egg production than hens fed grain concentrates (Zuidhoff et al., 1995).

While feeding roughage can reduce hunger motivation in some highly productive breeding animals, it may not always be sufficient to maintain welfare. Tina Widowski, an animal welfare specialist at the University of Guelph in Canada, has extensive experience in raising both breeding sows

and broiler breeder chickens. She told the first author that "broiler breeders are so highly selected for an appetite that they do not work behaviorally as animals." For example, when broiler breeders were fed ad lib., they spent 50% less time pecking objects (Sandilands et al., 2005). There is also some evidence that sows that produce large litters have more stereotypic behavior (Von Borell and Hurnick, 1990). D'Eath et al. (2009a) and Lawrence et al. (1989) both warn that feeding a bulkier, less nutrient-dense diet may not solve all the welfare problems associated with feeding motivation. Further research is needed on the effects of "metabolic hunger." There may be a point where the continued breeding of animals for appetite will cause grave problems.

Movement restriction versus feed restriction

The general public is often most concerned about movement restrictions when animal welfare is being discussed. Some examples are egg-laying hens housed in cages, veal calves housed in crates, and gestating cows kept in stalls where they are unable to turn around. Research clearly shows that animals are motivated for movement (Dellmeier et al., 1985; McFarlane et al., 1988). However, movement restriction may be less stressful to the breeding animals than feed restriction (Rushen, 1995; Rushen et al., 1993). This could be especially true in animals genetically selected for high appetite. In one experiment, gilts were housed in crates that made turning around difficult. In the first treatment, food and water were positioned at opposite ends of the crates so the gilts had to turn around to eat and drink. In the second treatment, the water and feeder were placed at the same end of the crate. Gilts that did not have to turn around did so just as many times as gilts that had to turn to eat or drink (McFarlane et al., 1988).

In another experiment, Dellmeier et al. (1985) found that veal calves housed singly in small stalls for six-and-a-half weeks responded with greater activity when tested in an open-field arena, compared to calves housed in groups in a yard. The calves housed in small stalls ran around and kicked up their heels when turned loose in the test arena. Calves raised in large group pens did not do this. Pasille et al. (1995) questioned the results of Dellmeier et al. (1985), because the open field was more novel for the calves housed in stalls. Pasille et al. (1995) suggest that Dellmeier et al. (1985) did not control for the novelty factor in the test arena. However, the first author has observed that cattle kept in a large, open feedlot pens and cattle housed singly in small "dog run" pens behave differently when released. Large, fat cattle raised in small "dog runs" ran up and down a 3.5-m-wide alley when released. The increased activity cannot be explained by the effects of novelty. Both groups of cattle were raised outside on the same farm and the alley was visible from their pens. Our informal observations support the findings of Dellmeier et al. (1985).

Nervous system abnormalities due to selective breeding for specific behaviors

The literature is full of examples of neurological or behavioral abnormalities in dogs, pigeons, mice, rabbits, and rats. The old research on pointer dogs is especially interesting (Dykman et al., 1979; McBryde and Murphee, 1974). It is important for modern breeders to be aware of the dangers of overselection for a behavioral trait. The first author recently talked to an acquaintance who hunts with a pointer. When he was informed about these old studies, he admitted that some breeders may still be making these same mistakes. Pointers are dogs selected to freeze and point when they see game birds hidden in the bushes. Pointing behavior is similar to an orienting response that becomes frozen. The selection of pointers for the pointing trait may be selection for an abnormal orienting response. Breeders have known for years that some dogs are so nervous that they are useless as hunting dogs (Dykman et al., 1979). The pointing trait and nervousness may be linked. A neurologically normal dog will orient toward a bird or other prey, then either chase it or return to its original activity. A normal dog does not stay frozen in an orienting posture. Breeders of pointers have recognized that a fine line exists that divides a good pointer from a bad one. Dogs with a heightened pointing response are generally too nervous to make reliable hunting dogs. Dykman et al. (1979) and Peters et al. (1967) found obvious differences in the behavior of normal pointers versus nervous pointers. Furthermore, visitors to the laboratory could easily differentiate which dogs were from the nervous genetic line. Nervous pointers cowered in their cages and failed to approach visitors, whereas the normal pointers approached and wagged their tails.

Geneticists have selectively bred both normal pointers, with the characteristic pointing behavior, and abnormal, nervous pointers. Nervous pointers are excessively timid and display a hyperstartle response, avoidance of people, and catatonic freezing in the close presence of people (Klien et al., 1988). Some nervous pointers display strikingly bizarre behavior. One dog became "frozen" at a point and fell over when accidently bumped by another dog (McBryde and Murphee, 1974). It appears that with nervous pointers, a continuum of neurological defects exists. Further research by Klien et al. (1988) revealed that many nervous pointers were also deaf. Inherited deafness in lines of pointers selected for excessive nervous behavior is an autosomal recessive trait, and the nervous trait may be inherited dominantly (Steinberg et al., 1994).

The welfare of normal pointers (ones without the nervous trait) is acceptable. However, nervous pointers may have very poor welfare unless raised under specialized conditions. For example, training and environmental modification can help nervous pointers act more like normal dogs. McBryde and Murphee (1974) found that training nervous pointers alongside normal pointers made them less timid. The dogs from the nervous genetic line become

less timid and followed a normal pointer. After a period of training, the nervous dogs no longer needed to be kept with a normal dog. McBryde and Murphee (1974) concluded that breeding an animal continuously for a certain genetic trait often resulted in the occurrence of other less desirable traits. In the 1990s, the Arkansas pointer lines were terminated. However, they contributed to our understanding of how genes and environment influence behavioral differences between dogs, including the development of potential therapies for shy or nervous dogs.

Inherited neurological defects

Arman (2007) states that the prevalence of inherited defects in dogs is too high. Breed associations should encourage selecting dogs for their functionality and allow the introduction of new genetics into the registered breeds. The list of neurological problems in dogs is huge (Ackerman, 2011). Belgium Malinois that has a circling disorder has genetic similarities to human obsessive–compulsive disorder (OCD) (Cao et al., 2021). Many examples of neurological defects in other animals can be found in the genetics literature. For example, roller-tumbler pigeons are bred to roll in flight. Pigeon fanciers select birds with an intermediate expression of this trait. Birds with an excessive expression of this trait continue rolling in flight until they crash into the ground (Entrikin and Erway, 1972). Pigeons that roll in flight have acceptable welfare, but when they hit the ground, welfare is severely compromised. In comparison to pointer dogs, a slightly abnormal orienting response makes a good bird dog, but an excessive amount of the trait results in a dog that is a nervous wreck.

Nervous defects in rodents and small mammals

It is important for researchers to read the descriptions of abnormal behaviors that are described in the old studies that are reviewed in this chapter. This is why we have kept them in the third edition. For hundreds of years, the Japanese have bred "fancy" mice as a hobby. In Europe, there is also a long tradition of mouse fanciers (Morse, 1978). Some fancy mice were selected for abnormalities of movement. Most of the fancy mouse mutations can be found in today's laboratory mice. One is called the "Japanese Waltzer." Cools (1972a,b) reported that these animals have an abnormality of the inner ear, are also hyperactive, and behave like mice injected with amphetamines (Cools, 1972a,b).

Researchers have bred rodents with many abnormalities such as seizure-prone rats (Seyfried, 1982; Serikawa and Yamada, 1986; Wimmer and Wimmer, 1985). Some rats have epileptic seizures triggered either by lowering their body temperature or by sound. Mutant, seizure-prone rats are so sensitive that a minor disturbance, such as changing drinking bottles, can

cause convulsions (Serikawa and Yamada, 1986). Others are generally prone to seizures, and many different types of stimuli can trigger a seizure. There are also strains of rodents with juvenile-onset or adult-onset susceptibility to seizure. Rabbits have also been selectively bred for susceptibility to sound-induced seizures (Hohenboken and Nellhaus, 1970). A preference for alcohol has also been genetically selected (Wimmer and Wimmer, 1985). McClearn and Rogers (1961) discovered a preference for alcohol over water in an inbred mouse line. Crabbe (1983) found two distinct genetic effects and two separate behavioral effects of alcohol. The first effect of alcohol on mice is on activity levels after a dose. The second is "hypothermic sensitivity," which includes a long sleep time after a dose of alcohol.

In rodents and small mammals used for research, welfare problems can appear unexpectedly. In a German laboratory, workers reported an outbreak of self-mutilation in checkered cross rabbits bred for a high resistance to infection (Inglauer et al., 1994). The rabbits bit off their own toes. The researchers achieved the goal of increased disease resistance, but the price was rabbits that injured themselves. This experience is just one more example of behavioral and physiological traits linked in ways that are difficult to predict.

Depigmentation patterns and the nervous system

For centuries, animal breeders have recognized that a lack of eye and body pigmentation may signal neurological defects. Animals with extensive depigmented areas on the body are more likely to have developmental or nervous system abnormalities (Cowling et al., 1994; Searle, 1968; Webb and Cullen, 2010). The degree of depigmentation could possibly serve as an early warning for welfare-related nervous system abnormalities in animals highly selected for production traits (Fig. 13.3).

It is important, however, to differentiate between depigmentation and animals with white hair and fully pigmented skin. Arab horses and Brahman cattle have light hair and dark skin. Depigmented areas of the body are characterized by both white hair and pink skin. The Holstein cow is depigmented in the white areas of the body. Light-colored cattle, such as the Charolais, are not depigmented and have brown eyes and some hair pigment. Most beef breeds do not have large areas of depigmentation. In addition, some crossbred white pigs with Duroc genetics have white hairs with a very faint reddish tint. The Yorkshire and Landrace pig breeds have dark-colored eyes and white hair and skin.

Coat color and patterns are controlled by a common genetic mechanism in many different mammals (Murray, 1988). For example, many domestic animals have a white blaze on the forehead and a white tail tip. Dogs, cats, and cattle are all found to have this pattern. Schaible (1969) and Schaible and Brumbaugh (1976) explain that migration of pigment-producing cells

occurs during embryonic growth, and common patterns between species frequently occur. For example, mice can be selected for a white belt on a mostly black body. This pattern is very similar in appearance to that of Hampshire pigs and Belted Galloway cattle. The common white areas in all three species are characterized by a lack of pigment, both in the hair and in the skin beneath the hair. In domestic horses, depigmented facial and leg markings were not present, or were very rare in their wild ancestors (Guerts, 1977). Woolf (1990, 1991, 1995) suggests that white markings on the face or legs provide less color protection from predators. However, flashy white markings are favored by some breeders, and Forbis (1976) reports that common white markings in the Arabian have been present since ancient times. Attractive white markings on the legs and forehead of horses are often deliberately selected. In contrast, depigmentation in production livestock and poultry is possibly a secondary effect of selection for production traits. Many domestic animals are partially depigmented, especially high-producing animals. A completely depigmented animal is an albino with pink eyes. A good example is white rats and mice. Several food-producing animals are partially depigmented and have either colored eyes or some areas of the body that are not white. The highest-producing pigs, milk cows, and chickens are either white or partially white. The maternal sow lines of commercial pigs are mostly white. High-producing commercially bred turkeys and broiler chickens have white feathers and white skin.

Depigmentation is both good and bad. Partial depigmentation in varying degrees is highly correlated with production traits. Partial depigmentation is also linked to behavior. The two calmest breeds of cattle, Herefords and Holsteins, have depigmented white areas on their heads. However, large areas of depigmentation often appear in conjunction with serious developmental or neurological problems. Many veterinarians report that mostly white Holstein dairy cows are more difficult to handle and more nervous than Holsteins with larger pigmented areas of hair and skin. Also, a genetic disorder called “white heifer disease” is commonly found in white heifers of the Shorthorn breed, or in breeds related to them (Rendel, 1952; Spriggs, 1946a,b). White Shorthorn heifers are usually sterile (Spriggs, 1946a,b). The ovaries and external genitalia are normal but the uterus and the vagina are not completely developed.

Depigmentation, behavior, and defects

There is definitely a relationship between depigmentation and behavior. When Belyaev (1979) selected foxes for tameness, the animals developed a piebald coat pattern with areas of depigmented white fur. Depigmentation of skin, hair, and eyes is related to the development of the nervous system. Searle (1968) found a relationship between depigmentation and deafness in several rodent species. In deer mice, Cowling et al. (1994) report a

relationship between deafness and the amount of depigmentation on the head. The mice also had ataxia (staggering) and retinal problems. Mice with the most extensive white areas were most likely to be deaf. Also in mice, the Variant-Waddler is a neurological mutant strain where individuals are hyperactive, deaf, and display an abnormal circling behavior. A remarkable relationship was found between asymmetrical spotted areas of fur and the preferred direction of circling (Cools, 1972a,b). This author postulated that asymmetry in the fur pattern and laterality in circling are expressions of the same basic neurological disturbance.

It is well known that white cats with blue eyes are often deaf (Bergsma and Brown, 1971; Schaible and Brumbaugh, 1976). In both dogs and cats, a white or piebald coat color is also related to an increased incidence of deafness (Schaible and Brumbaugh, 1976; Sorsby and Davey, 1954). Dogs that are vision or hearing impaired often have abnormal pigment loss in their coat and the iris of the eye (Savel and Sombe, 2020). Volpato et al. (2017) reported that piebald camels with blue eyes were often deaf, had visual impairments, and were difficult to handle. Bergsma and Brown's (1971) review of the literature discussed a continuum in the relationship between depigmentation and deafness. White cats with dark eyes or dark-pigmented areas of hair and skin on the head are less likely to be deaf, in comparison to white cats with blue eyes. Hudson and Ruben (1962) and Schaible and Brumbaugh (1976) both discuss deafness in Dalmatians, a heavily depigmented breed. Thirty percent of Dalmatians are deaf (Strain et al., 2009). Dalmations with the most extensive white areas are most likely to be deaf. In addition to Dalmations, other extensively depigmented dog breeds, such as pale-colored Australian shepherds have a high incidence of the brain, ear, and eye abnormalities (Schaible and Brumbaugh, 1976; Sponenberg and Lamoreux, 1985; Strain et al., 2009; Strain, 2011). A higher incidence of depigmentation and deafness is also found in albino cattle (Leipold and Huston, 1962). Paint horses can also have neurological abnormalities (Fig. 13.2). The authors speculate that selection for a moderate amount of depigmentation appears to provide production advantages, but excessive selection will cause serious welfare problems (Fig. 13.3).

Depigmentation is also related to abnormalities in the visual system. Guillery (1974) reports that many albino mammals have visual pathways with crossed connections to the brain. Siamese cats, which are partially albino, have crossed visual pathways (Guillery et al., 1971). The crossed visual pathways may not compromise the welfare of Siamese cats kept as pets because they adapt well to their miswired visual systems (Guillery, 1974). However, visual tests that were more demanding than shape discrimination revealed that albinos have subtle visual perception deficiencies. Sheridan (1965) found that black and white hooded rats with pigmented eyes performed better than albinos on a visual discrimination task with one eye covered. Compared to the albinos, the hooded rats performed the task better



FIGURE 13.2 These Japanese pigs seldom bite tails even though they live in an environment that is barren. Genetic factors have an influence on the occurrence of tail biting.



FIGURE 13.3 A paint horse with blue eyes has a nervous switch that resembles Tourettes (the disorder) in humans. Overselection for appearance traits is sometimes linked to neurological problems.

when they had to switch eyes. This principle is also vividly shown in mice. Guillery et al. (1971) found that flecked mice with variegated areas of both pigmented and depigmented areas of skin and fur have normal visual pathways to the brain, even though they are roughly 50% albino. The white areas of skin and hair are albino and the dark areas are pigmented. The welfare of laboratory albino rats is not compromised because they eat, drink, and mate normally. However, abnormalities of the visual system and auditory systems may cause functional problems and could possibly confound the results of

research on vision or hearing. Albino rats have more unmyelinated nerve fibers in the optic system compared to hooded rats (Lund, 1973). In albinos, the visual cortex is also thinner (Lund, 1973). This is a further indication of a correlation between nervous system abnormalities and depigmentation.

Highly depigmented or albino animals are rare in nature. When they do occur, the animals generally have difficulty surviving in the wild. One brief reference in *Science* by Minckler and Pease (1938) refers to a colony of albino rats living under feral conditions. These rats inhabited an area at a local dump in Montana. The exact source of these animals is unknown, but it was presumed that students from the local university released them. Abundant food, water, shelter, and a lack of predators created a sheltered environment suitable for an albino colony to survive. However, white cats depend on humans to maintain them. Even dark-eyed white cats do not survive under feral conditions. Furthermore, many animals with genetically based behavioral problems are often highly depigmented. For example, Viennese white rabbits are known to have seizures (Hohenboken and Nellhaus, 1970). In the paper on nervous pointer dogs by Dykman et al. (1969), both authors noticed that the five nervous pointer dogs in the photographs were almost all white. These dogs had greater areas of white fur than typical pointers. It would be interesting to measure the amount of depigmented areas on the bodies of both normal and nervous pointers and then compare these to Dykman et al.'s (1969) continuum of nervous traits.

The first author has observed possible signs of overselection for depigmentation in high-producing, commercially bred hybrid pigs. The nervous, excitable pigs discussed earlier are usually very lean and are white or mostly white. The relationship between depigmentation, albino genes, spotting genes, and nervous system problems is documented extensively in mammals (Arman, 2007; Silversides and Smyth, 1986). The link in poultry is much weaker. Harper et al. (1988) found that the "bobbler" defect in turkeys is not linked to feather color. The "bobbler" defect involves abnormalities in the inner ear and balancing difficulties. However, Silversides and Smyth (1986) found a relationship between lighter feather color and congenital tremor. The affected birds had abnormal myelin in the cerebellum of their brain and a reduction in melanin pigment in the eyes and feathers. A possible explanation for less of an effect in poultry might be due to the fact that baby chicks of the white poultry breeds are born with yellow down and adult white poultry have yellow-pigmented legs. The mutation that makes some of the feathers white in bronze-colored Leghorns is associated with weakness and the homozygous mutant does not survive (Somes, 1979). Photos accompanying this paper show that the partially white mutants also have very pale (almost white) feet and beaks as adults. Albinism and depigmentation in most mammals cause abnormalities in the rods in the retina that are used for night vision. However, albino birds do not have retinal abnormalities (Jeffery, 1997). Unlike most mammals, birds have a cone (color-vision)-dominant retina

and the development of the cones is unaffected. A few albino mammals, such as the squirrel, have relatively normal retina development because cones predominate (Jeffery, 1997).

Oskina et al. (2009) studied the involvement of glucocorticoid hormones in the appearance of white spots during embryogenesis. A delay in the migration and development of melanoblasts (pigment cells) in the embryos of fully pigmented gray rats was caused either by restraint stress (emotional) or by administering dexamethasone in drinking water. Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs. At days 12–14 of gestation, pregnant females were assigned to a treatment plan that consisted of being restrained in a small plastic cage for 45 minutes three times a day, or in the second treatment, dexamethasone was given to the pregnant rats in drinking water. In both treatment groups, three times more offspring were born with large areas of depigmented skin and hair on the ventral side of the body compared to the untreated control groups. It was also demonstrated that in adult offspring of mothers treated with dexamethasone, the response of the hypothalmo–pituitary–adrenal axis to emotional stress was lower. Prenatal emotional stress in fully pigmented mother rats caused a rise in glucocorticoid (stress hormone), which is associated with depigmented areas of skin and hair of the offspring. There is also a downregulation of the HPA-axis leading to a reduced response to stress in adult offspring. The same effects were shown by artificial administration dexamethasone (Oskina et al., 2009).

Fluctuating asymmetry and genetic problems

Another possible warning sign of both genetic problems and detrimental effects of the environment on development is the greater fluctuating asymmetry of an animal's body (Markow, 1992). Fluctuating symmetry (FA) refers to random deviations from bilateral asymmetry and can be measured in the body—as in bilateral symmetry of finger or foot lengths—or in a particular organ. FA is a key concept in evolution and development and underlies the theory of developmental stability—the ability to maintain normal development under stress. Animals with high levels of FA have reduced developmental stability. Deviations from perfect bilateral symmetry can be caused by environmental stress, or genetic problems during development, leading to developmental instability (Graham et al., 2010). Conservation biologists use measurements of body symmetry to assess the detrimental effects of environmental pollution in rainbow trout (Leary and Allendorf, 1989), chickens (Buijs et al., 2012; Erikson et al., 2003; Moller et al., 1995), and horses (Manning and Ockenden, 1994). Graham et al. (2010) discuss the importance of taking body measurements in several different body locations to get an accurate measure.

Fluctuating asymmetry is also affected by purely genetic causes. Parsons (1990) states that inbreeding increases the degree of asymmetry in a variety

of animals. The teeth of hybrid mice are more symmetrical compared to inbred mice (Bader, 1965). Symmetrical trout have greater heterozygosity and gene variation (Leary and Allendorf, 1989). Asymmetry also affects an animal's performance and adaptability. Manning and Ockenden (1994) measured the differences in the width and length of knees, teeth, ears, coronet bands, upper legs, and six measurements of the heads of racehorses. More symmetrical horses performed better on the racetrack. Symmetry is also involved in sexual selection. Moller (1992, 1993) found that female swallows preferred males with long, symmetrical tails.

There also appears to be a relationship between domestication and fluctuating asymmetry. Domestic animals tend to be more asymmetrical than wild animals (Parsons, 1990). Fluctuating asymmetry and minor physical abnormalities are signs of greater problems. Human children with developmental abnormalities such as mental retardation and autism have a greater percentage of minor physical abnormalities than the general population (Links et al., 1980; Steg and Rapport, 1975). They are also more likely to have greater asymmetry (Malina and Buschang, 1984). Assessments of body asymmetry may be one simple way to provide an early warning of potential problems with a selective breeding program. Intuitively, dairy cow breeders for years have evaluated various body parts for symmetry. For example, dairymen routinely look at traits such as a well-formed, symmetrical udder. All major dairy bull studs do this because they intuitively know that symmetry is a good trait.

An important conclusion can be made. Overselection for a single trait ruins the animal. In breeding animals, people must be aware of the complex interaction between traits that do not appear to be related.

Welfare and genetic selection

At what point has a genetic selection for a certain trait gone too far? Changing an animal's diet or housing conditions may provide decent welfare under some conditions, but not others. There may be a point at which the animal is so defective that it will suffer even when it is fed and housed in an ideal environment. If genetic selection for milk production goes too far, it is likely that Holstein cows may develop serious health problems that cannot be compensated for by improving their nutrition or housing. The first author calls this problem biological overload (Grandin, 2020).

Animals can be altered by genetic selection to such an extent that serious structural or neurological defects develop that can cause great discomfort. For example, animals with bowed legs or other structural abnormalities may have difficulty walking. Both authors agree that animal welfare is not acceptable if high-producing meat animals are chronically lame due to weak legs. An example of biological overload would be wooden breasts in broiler chickens. In the breasts of fast-growing chickens, there are areas of tissue

damage. In severe cases, a market weight broiler that falls on its back will not be able to right itself (Gall et al., 2019). Other examples of problems linked to genetic selection for production traits are congestive heart failure in cattle and leg conformation issues that can lead to lameness (Moxley et al., 2019; Cockram et al., 2019). An emerging possible welfare condition is high-producing dairy cows in udder swelling due to edema (Okkema et al., 2021). It is also our opinion that an adequate level of animal welfare is not possible if selected traits become so extreme that obvious mobility problems occur, or if the condition is known to be painful in humans. A good example of this is arthritis.

One may argue that measuring how an animal feels is very difficult. However, the nervous system of pigs and cattle has the same basic design as the human nervous system. Careful studies of different mammals reveal that surgical procedures do cause pain in animals (Molony et al., 1995; Short and Poznak, 1992). Noxious stimuli that cause pain in people are also likely to cause pain in animals (Molony et al., 1995; Short and Poznak, 1992). Both chickens and rats self-medicate to relieve pain by eating or drinking a bitter-tasting painkiller (Colpaert et al., 2001; Danbury et al., 2000). When a lesion in their joint heals, they stop ingesting the painkiller and switch to plain feed or water. This indicates that animals definitely feel pain (Danbury et al., 2000).

Both authors suggest guidelines for determining the ethical limit for genetic selection. Animals should have freedom from pain due to structural abnormalities. Overdeveloped meat-producing muscle that causes fatigue or an animal becoming nonambulatory during handling is not acceptable. Welfare is also seriously compromised in females unable to give birth naturally and forced to endure severe dystocias. There should also be low levels of behavior detrimental to welfare, such as hyperexcitability during handling or high levels of aggression. Some examples include heart attacks in pigs homozygous for PSS, hyperexcitable pigs that constantly back up in races and bunch and pile up at the slaughter plant, and nervous pointers that have difficulty adapting to normal dog environments. Another example is an inbred strain of mice that does not habituate to repeated exposure to a novel environment (Boleij et al., 2012). Since these mice lack normal habituation, their welfare may be severely compromised during standard laboratory procedures. To maintain an acceptable level of welfare, these mice need to be housed in conditions where exposure to sudden novelty is minimized. Severely compromised welfare can occur in these high-fear mice if not slowly trained to tolerate novelty. Selecting for excessive appetite motivation may cause stress, stereotypies, and other abnormal behaviors, especially in feed-restricted breeding animals. If stereotypies develop in large numbers of breeding animals housed and fed under the best conditions, genetic selection may have gone too far in the wrong direction. In some situations, a genetic line with low levels of repetitive stereotypies had higher-fear temperaments

(Hansen and Jeppensen, 2006; Svendsen et al., 2007). Research has shown that relationships between temperament and high and low stereotypies are not simple. Minks showing low fear when fear was measured by willingness to explore a stick stuck in their cage may become very frightened when fear is measured with a different method. Minks with high levels of stereotypies had higher fecal cortisol metabolites after they were immobilized (Malmkwist et al., 2011). High stereotyping minks had more kits, but the low stereotyping minks had fewer, better-quality kits (Meagher et al., 2012). Maybe the high stereotyping minks were high "seekers" and more willing to approach novelty (see Chapter 1). Malmkwist et al. (2011) conclude that minks with high stereotypies may be more sensitive to stress. An animal demonstrating continuous stereotypies has severe difficulty with basic functioning.

Siamese cats with mildly crossed eyes have good welfare, but animals bred to have constant epileptic seizures may have poor welfare if not housed in an environment free of seizure triggers. It is clear that animal welfare problems have occurred in some animals as a result of overzealous selection for a single trait. Many dog breeds have structural problems that compromise welfare (Ackerman, 2011; Arman, 2007; Ott, 1996). Some examples are eye problems in collies, back problems in dachshunds, breathing problems in bulldogs, and hip problems in many breeds. Many of these traits can be selected without compromising welfare if the selection is done in moderation. Animal breeders need to take a more holistic view when selecting breeding stock and think about optimizing the whole animal instead of selecting for a few traits. This would help prevent the mistakes made by pig breeders discussed earlier in this chapter. Pig breeders selected for production traits forgot to examine animals for structural soundness and failed to see the leg problems until half of the pigs became lame.

The science of genetic engineering now makes it possible to delete genes or swap genes between species. A major difference between conventional selective breeding and genetic engineering is that the process of selection is greatly sped up (Fox, 1989, 1992; Grandin, 1991; Rollin, 1995). The speed of change is accelerated to a point where less time is available to take corrective action if selection mistakes are made.

Transgenic animals used to study human neurological disorders

Genetic engineering, or conventional breeding, is used to make animal models for various human diseases by either inserting genes into animals or by highly selective breeding. Since the publication of the second edition, there are now many more transgenic animals. There are mini pigs that have the gene for Huntington, a severe brain disorder in people (Rieke et al., 2019), rodents with genes for neurodevelopmental in humans, and differences in

aggression in transgenic animals (Jager et al., 2018). There are hundreds of genetically engineered mice for use as animal models for human neurological conditions (Crawley, 2007; McFarlane et al., 2007; Wellberg, 2011; Walberg, 2011). For example, mice that have autistic-like behaviors can be purchased from a commercial company. The "autistic" mice have increased repetitive behavior, such as self-grooming (McFarlane et al., 2007). To avoid a possible welfare problem in this animal model, the mice should be carefully monitored to ensure that excessive self-grooming does not turn into self-injurious behavior. Mice altered by removing a gene involved in the regulation of serotonin display behavior that resembles OCD in humans. The mice altered by genetic engineering methods ran around repeatedly in the same alley of a maze. Normal mice will quickly try a new alley (Chen et al., 1994). Hooper et al. (1987) created a mouse model for Lesch-Nyhan syndrome. Children afflicted with Lesch-Nyhan syndrome self-mutilate and bite themselves. If this model is developed to the point at which the syndrome is fully expressed, the welfare of the mice will be severely compromised (Rollin, 1995). Transgenic cattle and pigs are also being used to produce substances useful in human medicine (Murray et al., 2010). For readers interested further in this topic, there is an excellent illustrated book to help technicians monitor the health and welfare of mice, rats, and rabbits (Pritchett-Corning et al., 2011).

It is beyond the scope of this chapter to discuss all the ethical arguments about genetic engineering, but there is a greater ethical justification to cause some suffering in a mouse if the mouse model makes it possible to cure Lesch-Nyhan syndrome. This syndrome is one of the most horrible genetic conditions that can affect a child. It is the opinion of both authors that causing pain to make a pig grow slightly faster is absolutely not acceptable.

Unexpected linked traits

Genetically altering an animal by knocking out selected genes can affect the behavior of the animal in unexpected ways. Researchers and commercial companies using biotechnology should be careful and not forget that changing a targeted piece of genetic code can have unexpected effects. Researchers are cautioned to avoid repeating the mistakes we reviewed in the first edition of this book. For example, Silva et al. (1992) used genetic engineering methods to knock out the gene that encodes for a substance called calcium/calmodulin-dependent kinase II. This substance is involved in the mechanisms of learning. The "knock-out" mutant mice displayed impaired spatial learning when tested in a water maze that required them to find a platform hidden under the surface of milky-colored water. The impaired spatial learning was similar to that of normal mice with hippocampus lesions. The hippocampus is a brain structure crucial for forming memories. However, Silva et al. (1992) found that knocking out this gene affected

more than just learning: the knock-outs also had an abnormally enhanced acoustic startle response and were more likely to jump when they heard a sudden loud noise. In 1994 researchers in another lab made a startling discovery of these same knock-out mutant mice. Mice were sometimes found dead in their cages with broken backs. The fighting behavior of these mice was so extreme that they suffered broken backs (Chen et al., 1994). This is another example of an unexpected effect. Knocking out the gene for calcium/calmodulin kinase II affected more than just learning: it also completely removed fear causing uninhibited fighting. Calcium/calmodulin kinase II is also involved in the regulation of the neurotransmitter serotonin. The knock-outs had normal behavior except in the area of defensive aggression. Defensive aggression was measured by quantifying attacks by a resident mouse against a mouse that was placed in its cage. Offensive aggression was measured by determining how hard an intruder mouse fought a resident. Since the knock-outs had no fear, they continuously attacked a resident mouse when placed in its cage. The heterozygote knock-outs with one knock-out gene had abnormally high defensive aggression (Chen et al., 1994). Offensive aggression, which is attack aggression, was attenuated in the genetically engineered mutants.

Researchers in another laboratory were also surprised by unexpected results when a specific gene was blocked. Giros et al. (1996) found that blocking the effects of the dopamine (neurotransmitter) gene in mice had both predicted and unexpected results. Blocking the effect of the gene made the mice produce an overabundance of dopamine in their brains. Dopamine excess is believed to be responsible for some of the symptoms of schizophrenia in people. As expected, homozygous mice had two copies of the blocked gene were hyperactive and ran around more compared to the heterozygous (one copy of the gene), or normal, wild-type mice. Neither type of genetically altered mice had stereotypies. An unexpected finding was that injections of amphetamines had no effect on the homozygotes, and an eightfold increase in locomotor activity in the heterozygotes and normal, wild-type mice (Giros et al., 1996). This experiment provided important insights into how pharmaceuticals work and showed that the effects of drugs on behavior are not simple and straightforward. Similar effects of fear may operate in mice bred and selected by conventional methods. Male mice that were bred to have heightened reactivity to stimulation were less aggressive (Gariepy et al., 1988). Since the first edition of this book, researchers have developed more sophisticated behavioral tests for use with all the new mouse models (Crawley, 2007).

Speculations of genetic engineering

What if, through the use of genetic engineering, an animal was created that would feel no pain and have no fear? What would be the ethical implications

of this? The work of Chen et al. (1994) showed very clearly that knock-out mice with no fear, when mixed with unfamiliar mice, caused serious welfare problems due to fighting and injury.

In contrast, selecting extreme docility with conventional breeding methods could cause similar problems. Ranchers have observed that placid Hereford bulls spend more time fighting with other bulls to determine their social rank compared with flighty, excitable Salers bulls. The more excitable animals are too fearful to fight. The second author has made similar observations about horses. The calmest horse on a pasture is more likely to bully the other horses. Timid horses avoid a confrontation that could lead to injury. There may be an optimum temperament between extreme docility and flightiness. Motivation is complex. In Chapter 11, Faure's research shows that fear is not a simple trait. Fear behaviors may look similar but have different motivations. For example, feather pecking in hens is probably misdirected foraging behavior instead of true aggression. Cattle and pig breeders need to select for a calm temperament and cull individuals that panic when confronted with novelty, but it may be a mistake to select for the absolutely calmest animals. An extremely docile cow may not take care of her calf (see Chapter 5). In the Chen et al. (1994) experiment, increased defensive aggression only occurred in mice heterozygous for the knock-out gene. Mice with both calcium/calmodulin kinase II genes knocked out had overall attenuated aggression and many other abnormal behaviors. The behavior in these mice differed depending on whether one copy or both copies of the gene were knocked-out. This research clearly shows that knocking out genes is not going to provide clear-cut results. As with natural breeding, traits are linked. The behavior of an animal is the sum of a complex interaction between inherited traits and those traits' interactions with the environment.

Genetic diversity

Another serious concern brought up by ethicists and geneticists is the problem of restricting the gene pool. The American Holstein has a narrow gene pool. Most of the cows are sired by two bulls (Charles, 2019). This problem is not limited to genetic engineering. Many scientists are concerned about the loss of genetic diversity (Notter, 1996; Cundiff et al., 1996). Larry Cundiff, a researcher who works for the US Department of Agriculture, stated that pig breeds in China are more heterozygous. Preserving the genetic diversity of Chinese pig breeds is important.

The loss of genetic variability in agricultural plants and animals could result in disaster if disease struck a susceptible high-producing genetic line. Nothing is free. If one selects just one trait, there is usually a price to be paid by weakening another trait. In 1977 the American Livestock Breeds Conservatory was founded to preserve rare breeds of domestic livestock and poultry. However, some agriculturists do not see the wisdom of keeping

older and lower-producing breeds of pigs and chickens. The reason for conserving older breeds is they retain desirable traits such as disease resistance, fertility, and hardiness (Thomas, 1995; Sponenberg, 1995; Wainwright et al., 2019).

The more specialized a domestic animal becomes, the more specialized an environment it will require. A Holstein cow requires more environmental support from humans than a beef cow. Beef cows and horses can still go feral and survive under wild conditions. The first author has observed feral cows on mountain ranches that were able to avoid being rounded up for up to 10 years. The Holstein milk cow, with her huge udder, would have difficulty living under natural conditions. In addition, the first author has observed that Holstein calves are weaker and take longer to walk through races and chutes compared to beef-breed calves.

In the plant kingdom, corn is an example of a totally domestic plant. It cannot grow without assistance from humans. Modern corn is radically different from its ancestor, teosinte. Teosinte had hard kernels covered with an inedible shell, whereas modern corn has more kernels and no hard shell over them. Research by Jane Dorweiller at the University of Minnesota showed that converting teosinte into modern corn involved changing just a small stretch of DNA. Plants have been modified more by domestication than animals.

Ethical questions

One may speculate, what if a farm animal breed was manipulated as much as corn has been? Modern corn breeds look like a different species compared to wild corn. Would it be ethical to create microcephalic cows with almost no brain? Fox (1989, 1992) was the first to ask this question. If the nervous system was modified so the mutant would not suffer, would it be ethical? Philosophers can fight over this one.

Being practical people with years of hands-on experience with animals, both authors agree that decisions on the ethical use of biotechnology should be based on the concept of ethical cost. Invasive or painful experiments or the creation of animals with chronic pain should not be done for frivolous reasons. It may be justified to cause some pain or discomfort in an animal to find a cure for multiple sclerosis, but it would not be morally justified to make animals suffer to grow larger amounts of meat or produce a few more kilograms of milk.

Furthermore, it is our opinion that extremely fearful or aggressive agricultural animals are not acceptable from an animal welfare standpoint. We are also very concerned about the welfare of breeding animals selected for increased appetite. In the laboratory, genetically altered animals can provide tremendous knowledge about both genetics and the nervous system if researchers take a few simple precautions, most mutants can be maintained

in a laboratory with good welfare. The "no-fear" mice would have an adequate level of welfare if caretakers made sure not to mix unfamiliar mice together. However, homozygous mutants with hyperlocomotor activity may be more compromised. Breeding large numbers of these mice may not be ethically justified, but breeding in small numbers may be justified to learn about the mechanisms of neurotransmitter pathways and that could lead to the development of new medications and treatments for many serious disorders. A researcher sensitive to the welfare of the animals in his laboratory could maintain mutant breeding stock with large numbers of heterozygotes, which have more normal behavior, and keep just enough homozygotes for ongoing experiments.

The farm versus the laboratory

A big difference exists between hundreds of mice in a laboratory and thousands of pigs or chickens used for the commercial production of animal protein. As animals on commercial farms often are not managed as carefully as research animals, genetic characteristics causing minor welfare problems in a controlled laboratory setting could cause horrible suffering on a farm. This would be especially true if the farm was poorly managed. If a "no-fear" pig was developed, on-farm welfare could be deplorable because the animals might seriously injure each other during fights. New research shows that fear is not a single trait. Different types of fear are controlled by different brain circuits (Gross and Canteras, 2012). Selective removal of different fear pathways could have different effects. On the other hand, if aggression was totally removed to eliminate fighting, new problems might occur. Maybe a fearless, totally nonaggressive cow or sow would fail to take care of her babies, or totally nonemotional mutants would be too lazy to fully graze a pasture. The authors conclude that one should be more cautious when genetically altering an agricultural animal compared to a laboratory animal. Even when animals are bred naturally, one must be careful to ensure that the animal fits into its environment. LeNeindre et al. (1996) found that cattle and sheep bred for intensive conditions with daily close contact with people may have behavioral problems when housed extensively. For example, gentle cows under intensive conditions may become aggressive toward humans when reared on large pastures where they seldom see people. This raises the question: is it preferable to engineer animals to fit industrial systems, or rather to engineer systems that fit the animals in the first place?

Thirty years ago, the first author was asked to present a paper on biotechnology and meat production (Grandin, 1991). In this paper, a brief history of science was reviewed. Scientists in the past were persecuted and sometimes killed for discussing and discovering forbidden knowledge. Yesterday's forbidden knowledge is today's accepted fact. Galileo was persecuted for writing that the Earth was not the center of the universe. One should remember,

in the past, medical knowledge was held up for a thousand years due to the prohibition against dissection. The editor of *Science* suggested that we must proceed with biotechnology, but we should proceed with caution (Koshland, 1989). Biotechnology has proceeded. Genetically modified corn and soybeans are routinely used around the world, but biotechnology is still controversial. The first genetically modified salmon was sold for food in Canada (Waltz, 2017). These fish grow much faster. There are now hundreds of genetically modified mice that are used as models in research on cancer, autism, and many other disorders (Lutz, 2018; Meyza and Blanchard, 2017; Kersten et al., 2017). For the third edition, it was difficult to find recent studies that describe possible behavioral problems with these mice. A study by Queen et al. (2020) demonstrated that environmental enrichment improved social behaviors in a mouse model for autism. Genetic modification can also be used to improve animal welfare. A genetically modified pig can be protected from swine fever, a fatal disease (Xie et al., 2018). Genetic editing could be easily used to eliminate horns on cattle and the animals would no longer have to endure painful dehorning.

For this third edition, it is still appropriate to end this chapter with the last two sentences of the first author's paper given in 1991.

We should proceed cautiously, but we should definitely proceed. Biotechnology can be used for noble, frivolous, or evil purposes. Decisions on the ethical use of this powerful new knowledge must not be made by extremists or people motivated purely by profit.

There will be big questions in the future. While finishing the revision of this chapter for the third edition, the first author learned about the work of Christopher Mason. In his book, The Next 500 years, he proposed genetically modifying humans to better withstand the rigors of space on other planets (Mason 2021; Szocik, 2021). The statement the first author made more than 30 years ago will be even more important.

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