5 Genetics of dog behavior

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5.1 Introduction

Selection for behavior has played a key role in the history of dog domestication and breeding. Early dog domestication probably involved selection for tameness. A few generations of selection for tameness in the famous Russian silver fox experiments led to a domesticated strain of foxes that showed dog-like behavior and displayed curly tails, drop ears, and loss of pigment (Trut *et al*., 2009). The early domestication of dogs was followed by the formation of dog breeds. Different aspects of ancestral wolf behavior have been selected for in different breeds: dogs have been bred to guard, herd, hunt, pull sleds, and to provide companionship. Selection for physical appearance became more important at a later stage when people began breeding dogs for show. Extreme population bottlenecks,[1] founder effects,[2] drift[3] and strong artificial selection for desired traits during breed formation have resulted in a dog population that is a collection of genetic isolates with highly diverging morphology, disease susceptibility, and behavioral characteristics (Sutter & Ostrander, 2004).

Scott and Fuller (1965) performed a pioneering study of breed differences and inheritance of canine behavior. Their experiment involved dogs of five breeds: basenji, beagle, American cocker spaniel, Shetland sheepdog and wire-haired fox terrier. Breeds and their crosses were compared for reactivity, trainability and problem-solving behaviors. Scott and Fuller observed behavioral differences between the breeds in the majority of their behavioral tests; for instance, in playful aggression and dominance. Wire-haired fox terriers were the most aggressive, consistently "ganging up" on group members. These attacks were so serious that victims had to be removed in order to prevent serious injury. More recently, Svartberg (2006) compared the behavior of 31 dog breeds using data from a standard behavioral test. Significant breed differences were observed for all investigated traits (Figure 5.1). Dog breeds also differ in the prevalence of problem behavior. For instance, certain breeds are predisposed to obsessive-compulsive behaviors: bull terriers frequently exhibit tail chasing, while Doberman pinschers are prone to acral licking.

The fact that breed differences in behavior exist, and that behavioral dispositions can be selected for, suggests that there is a genetic basis for behavior. Behavioral genetics is the study of the individual variation in behavior due to genetic differences between animals. Behavioral genetic studies in dogs have traditionally been studies of breed differences, selection studies, and population-based heritability studies. More recently, researchers have attempted to identify genetic variants that explain behavior at the molecular level. This chapter reviews both the earlier heritability studies and the more recent molecular genetic studies of behavioral traits in dogs.

[1]A population bottleneck is a marked reduction in population size followed by the survival and expansion of a small random sample of the original population. Many dog breeds experienced bottlenecks at times of war and economic depression.

[2]When a species invades a new area, the original, small population is called a founder population. The term is usually applied in a context of subsequent population growth. Recent founder populations exhibit reduced genetic variation due to the population bottleneck. Many dog breeds were established from a small number of founders. Genetic homogeneity is maintained in dogs by the "breed barrier rule," i.e. no dog may become a registered member of a breed unless both its parents are registered members of the same breed. In other words, dog breeds are genetic isolates.

[3]Drift refers to changes in allele frequency over generations due to random factors. It leads to lower genetic variation, especially in small populations. All dog breeds have been subject to drift.

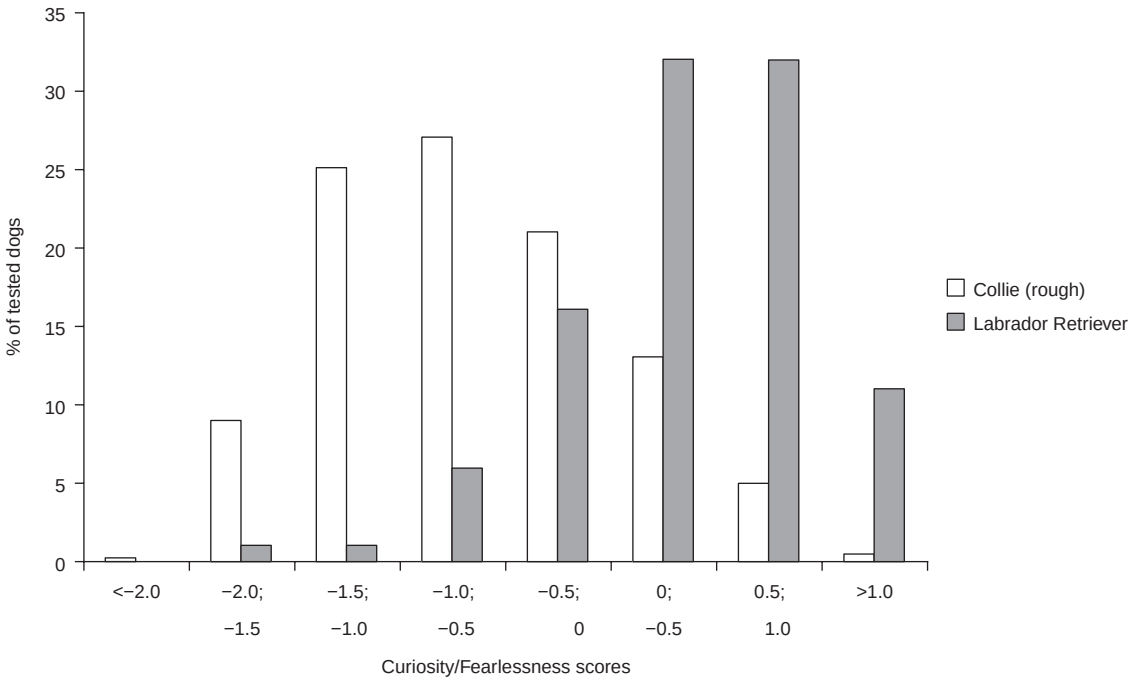

Figure 5.1 Results of the study of breed-typical behavior (Svartberg, 2006). The distribution of curiosity/fearlessness scores in the Dog Mentality Assessment (DMA) behavioral test is shown for two dog breeds: collies and Labrador retrievers. Labradors were the highest ranking breed for this trait and collies ranked the lowest. Despite substantial within-breed variation, the means of the distributions of the two breeds are shifted with respect to each other. (Figure reprinted from Svartberg (2006) with permission from Elsevier.)

5.2 Quantifying the genetic influence on behavior: Heritability

5.2.1 Principles of heritability studies

People that use dogs as working animals are obviously interested in knowing the degree to which behavioral variation is correlated with genetic variation. Heritability (h^2) is a measure of the relative contribution of additive genetic effects[4] to the total variance observed in a trait (Bourdon, 1997; Nicholas, 2003). It can also be described as the proportion of the parental deviation from the population average that is transmitted to the offspring. Total variance in a trait is comprised of a genetic part and a non-genetic part. The latter arises from environmental effects and is often called environmental variance or residual variance.[5] Heritability estimates are ratios and can thus

[4]The term "additive genetic effects" refers to the effect of independent genes. Non-additive genetic effects are gene combination effects, i.e. dominance or epistasis.

[5]Environment in behavioral genetics refers to all factors other than inherited factors. Examples in dogs include prenatal circumstances (such as stress in the pregnant bitch) and early experiences such as maternal care (Scott & Fuller, 1965), living conditions at the home of the breeder (Appleby *et al*., 2002), illness as a puppy (Podberscek & Serpell, 1997), and socialization (Appleby *et al*., 2002; Houpt and Willis, 2001; Scott & Fuller, 1965; see also Serpell *et al*., Chapter 6).

be influenced by changes in environmental variation. For instance, h^2 estimates will increase if animals are assessed in a consistent manner because this will lead to reduced experimental variation. The estimates also often differ between male and female dogs. Strictly speaking, h^2 estimates only apply to the population from which they were derived in the time period when they were assessed. In spite of these limitations, h^2 estimates can provide a broad guide to the relative contributions of genetic and environmental influences on population variance of a trait. Heritabilities may predict the consequences of various selection procedures. Highly heritable traits are expected to respond well to direct selection and performance testing. Traits with a low h^2 may require progeny selection[6] or the use of crossbreeding to produce genetic advantages.

Heritability estimations are based on resemblances between relatives. Family, adoption, and twin designs have been used to estimate the h^2 of behavioral traits in humans (Boomsma *et al*., 2002). Such studies have shown that there is a significant genetic influence on the majority of human behaviors examined. Heritability estimates for some mental disorders in humans are very high, e.g. 0.90 for autism (Burmeister *et al*., 2008). Older heritability studies in dogs often used paternal half-sib correlations, i.e. correlations between the performances of different progeny sired by a particular father.[7] Only the performance of the progeny is required, not that of the sire. An alternative method is offspring-parent regressions, using either one parent (usually the sire) or the average of both parents (termed offspring-midparent regressions). The performance of both offspring and parents are used in these types of study. Modern heritability studies use data about all available individuals by applying Best Linear Unbiased Prediction (BLUP) techniques (Henderson, 1975). In BLUP, breeding values of individual dogs are calculated based on phenotypic information on the dog itself, its parents, siblings, half-siblings, its progeny, as well as more distantly related dogs. Several reviews discuss the heritability of behavioral traits in dogs, particularly in relation to working behavior, temperament, and behavior problems (Hall & Wynne, 2012; Houpt & Willis, 2001; Hradecká *et al*., 2015; Mackenzie *et al*., 1986; Ruefenacht *et al*., 2002). Heritability estimates for canine behavioral traits are mostly in the low to moderate range. This is no surprise because traits that have undergone selection in the past are expected to show reduced additive genetic variance (Fisher, 1930).

5.2.2 Heritability of canine working behavior

Dogs were originally selected for working behavior, e.g. herding, guarding, hunting, and pulling sleds. As a result, retrievers retrieve and pointers point. The results of selection for working behavior can also be observed in the canine genome. For instance, Huson *et al*. (2010) observed that the genetic profile of Alaskan sled dogs bred for sprint racing differs from that of Alaskan sled dogs bred for running long-distance races. Many publications present heritability estimates of performance in canine working tests (see Ruefenacht *et al*., 2002; Hall & Wynne, 2012 for reviews). In most of these studies, large numbers of dogs were exposed to a range of standard situations, and their behavior scored by trained test leaders. Heritability estimates for these kinds of scored traits are mostly in the low to moderate range, e.g. between 0.09 and 0.24 in the German shepherd field behavior test (Ruefenacht *et al*., 2002), between 0.1 and 0.4 in the flat-coated retriever hunting behavior test (Lindberg *et al*., 2004), between 0.14 to 0.50 for herding behavior in border collies (Arvelius *et al*., 2009), between 0.07 and

[6]Progeny selection indicates choice of breeding animals based on phenotypes of their offspring. This differs from direct selection, where breeding animals are chosen based on their own phenotype.

[7]This is typically a study design with several sires that are each mated to several different dams. Progeny of a single dam are full siblings (sibs). Progeny produced by different dams, but from the same sire, are half-siblings.

0.18 for defense abilities in Belgian shepherd dogs (Courreau & Langlois, 2005), and between 0.15 and 0.32 for service dog performance (Wilsson & Sundgren, 1997a). For some traits, higher heritabilities were found. For example, "waiting passively in a group" in the hunting behavior test of the Swedish flat-coated retriever club had an estimated h^2 of 0.74 (Lindberg *et al*., 2004).

5.2.3 Heritability of dog personality

Personality or temperament can be defined as "underlying behavioral tendencies that differ across individuals, that are consistent within individuals over time, and that affect the behavior that is expressed in different contexts" (Stamps & Groothuis, 2010, p. 302). The Swedish dog mentality assessment test (DMA) is designed to provide insight into canine personality traits. The DMA is a standardized behavioral test that consists of 10 subtests, during which dogs are exposed to several different situations. The reactions of the dogs are recorded for 33 behavioral variables. Svartberg & Forkman (2002) collected DMA data from 1175 dogs of 47 breeds. The investigators performed factor analysis[8] on these variables and extracted five primary factors: (1) playfulness; (2) curiosity/fearlessness; (3) chase-proneness; (4) sociability; and (5) aggressiveness. Svartberg (2006) analyzed DMA scores from 13 097 dogs of 31 breeds and reported substantial breed differences in personality (Figure 5.1). Higher order factor analysis showed that all factors except aggressiveness were related to each other, creating a broad factor that influences behavior in a range of situations. This broad factor is comparable to the shyness-boldness axis in humans, which reflects a tendency to approach novel objects and a willingness to take risks (Wilson *et al*., 1994). This broad dimension is supported by the results of earlier dog studies, and a similar dimension was also detected in silver foxes (Kukekova *et al*., 2011a). It was found to be related to performance in working tests (tracking, searching, delivering messages, handler protection): high-performing dogs were bolder than low-performing dogs (Svartberg, 2002). Saetre *et al*. (2006) estimated the heritability of the DMA behavioral traits using 10 000 German shepherd and Rottweiler dogs that all completed the DMA. Their results suggest that there is shared genetics behind all behavioral traits except in those related to aggression. Heritabilities were generally low, ranging from 0.04 (remaining fear) to 0.19 (tug-of-war). The heritability of shyness–boldness was estimated to be 0.25 in these two different breed samples. Several smaller heritability studies have used measures other than the DMA for personality traits. Ruefenacht *et al*. (2002) reviewed these studies and reported that heritability estimates for traits related to canine personality range from 0 to 0.58 with an average of 0.20.

5.2.4 Heritability of problem behavior

Canine behavioral problems such as aggression, anxiety and obsessive compulsive behaviors pose serious threats to canine welfare. Behavioral problems accounted for 24% of dogs euthanized in Danish veterinary practices in a study of Mikkelsen and Lund (2000). Problem behavior is usually normal behavior manifested in extreme or inappropriate ways. An example is fearfulness. Fear is an instinctive natural response to a threat. For the majority of dogs, fear is of short duration and increases the chance of escaping danger. For dogs with an anxiety disorder, the state of fear lasts much longer and the dog may become extremely sensitive to any perceived threat. The most

[8]Factor analysis is a statistical method that aims to reduce a large number of variables to a smaller number of underlying variables (factors) based on the pattern of correlation between the variables.

common anxiety disorders in dogs are separation anxiety, noise phobia, thunderstorm phobia, and generalized anxiety (Bamberger and Houpt, 2006). Selection experiments and studies of breed differences suggest that a genetic component plays a role in the development of these disorders. For instance, Scott and Fuller (1965) found that all basenji puppies showed some fear response during a handling test compared to only 38% of cocker spaniel puppies. Another example is the work with nervous pointer dogs involving two collaborating research groups in Arkansas, USA. They established two breeding lines of pointers that were maintained for decades (Dykman *et al*., 1966). One line showed extreme fearfulness and avoidance of novel stimuli. The other was a stable control population. The nervous pointer dogs are considered a genetic animal model of severe anxiety. Goddard & Beilharz (1982; 1983) published papers on the heritability of traits that determine the suitability of dogs as guide dogs for the blind. Fearfulness was the most frequent cause of failure of potential guide dogs. It was measured with a behavioral test, scoring nervousness, suspicion, sound shyness, and anxiety. Fearfulness was estimated to have a heritability of 0.46–0.5 in this population of dogs (Goddard and Beilharz, 1982; 1983).

Aggressive behavior is also related to guide dog failure (Takeuchi *et al*., 2009a). Aggression is relatively well studied in dogs because of its serious implications for the bond between dog and owner (De Keuster *et al*., 2006; Landsberg, 2004; Mikkelsen & Lund, 2000). Like fearfulness, canine aggression is usually normal behavior (Borchelt and Voith, 1996; Mills, 2003; Mugford, 1984; Reisner, 1997). Wolves use aggression to defend themselves from predators and to compete for food, territory, social status, or reproductive opportunities. Situations that elicit aggressive behavior in wolves can also elicit aggression in dogs. There are several kinds of aggression and the subtypes seem to have a distinct genetic basis (Lesch and Merschdorf, 2000; Popova *et al*., 1993; Yeh *et al*., 2010). Some breeds have intentionally been selected for aggressiveness. For instance, Rottweilers, Dobermans, and German shepherds have been selected for stranger-directed aggression and American pit bull terriers have been selected for fighting purposes (Lockwood and Rindy, 1987; see also Lockwood, Chapter 9). There is substantial scientific evidence for breed differences in aggressiveness (e.g. Beaver, 1993; Blackshaw, 1991; Borchelt, 1983; Bradshaw *et al*., 1996; Duffy *et al*., 2008; Hart & Miller, 1985; Landsberg, 1991; Wilsson & Sundgren, 1997b). Some early studies of aggression-related traits in police or military dogs failed to produce h^2 estimates higher than zero (Reuterwall & Ryman, 1973; Willis, 1976). However, this is probably due to methodological problems (Houpt & Willis, 2001; Mackenzie *et al*., 1986). Heritabilities of aggression in the DMA were low but significant: between 0.06 and 0.12 (Saetre *et al*., 2006). Courreau & Langlois (2005) reported heritabilities of 0.14–0.16 for aggressive behavior in defense competitions of Belgian shepherd dogs. Pérez-Guisado *et al*. (2006) obtained an h^2 estimate of 0.33 for dominant-aggressive behavior in the Campbell test. It thus seems that the heritability of aggression is low but significant in the general dog population, although it may be much higher in specific dog populations (e.g. Liinamo *et al*., 2007).

5.3 Finding the underlying genes: Behavioral gene mapping

5.3.1 Principles of gene mapping

People are also interested in pinpointing the genetic variants that explain the inheritance of behavioral traits, i.e. gene mapping. Such molecular genetic information may facilitate the development of genetic tests that can be applied in dog breeding. Gene mapping is based on the assumption that

a mutation that affects protein structure or level occurred in the germline of an ancestral dog. Such a mutation is referred to as the causative (or causal or functional) variant. Scientists use flanking polymorphisms[9] to determine the position of the causative variant in the genome. Examples of such polymorphisms are single nucleotide polymorphisms (SNPs[10]), variable number of tandem repeats (VNTRs[11]), and short interspersed nuclear elements (SINEs[12]). The polymorphisms are referred to as polymorphic markers in this context.

If the trait of interest is monogenic (i.e. variation in the trait is influenced by variation in a single gene), we expect a simple relationship between causative variant and trait, e.g. all Labrador and golden retrievers with a premature stop codon in the melanocyte-stimulating hormone receptor gene have a yellow coat (Everts *et al*., 2000). Such variants can be mapped using linkage analysis in dog pedigrees (e.g. Acland *et al*., 1998). In complex traits such as behavior, a single mutation is likely to have only a small effect on the trait (see Box 5.1). The relationship between causal variant and trait is then diluted by variation in other genetic factors and the environment. Linkage analysis is less suitable in this situation. Association studies are used for mapping the polygenes involved in complex traits. In association studies, genotypes are correlated with a quantitative trait (quantitative trait association study) or allele frequencies are compared between cases and controls (case-control study design).

Association studies can focus on candidate genes or apply a genome-wide approach. Candidate genes are chosen based on pre-existing data derived from pharmacological, physiological, biochemical, anatomical, or knockout mouse studies. In behavioral genetic studies they often involve brain neurotransmitter systems. The selection of candidate genes is necessarily based on limited understanding of the biological pathways involved in the trait. Further progress in behavioral trait mapping is expected with genome wide association studies (GWAS). In a GWAS, the complete genome is scanned for association with a trait in multiple unrelated individuals using a large number of SNPs. Here, there is no *a priori* assumption about which genes are involved in the phenotype. This opens the opportunity for finding genes that have not been associated with behavior to date. Such analyses have only recently become possible because of the sophisticated genomic resources and the large number of subjects required.

[9] A polymorphism is the occurrence of two or more versions of the same DNA fragment in a population. In other words, a polymorphism is a variation in DNA sequence from a reference sequence. A polymorphism is the result of a single random mutation which has spread through the population by inheritance. Note that the difference between a mutation and a polymorphism is their population frequency: when the frequency of a mutation rises above 1%, it is referred to as a polymorphism. The general term "genetic variant" refers to both mutations and polymorphisms. A polymorphism can be neutral or it can affect protein structure or level.

[10] A SNP is a DNA sequence variation where a single nucleotide differs between individuals. Millions of SNPs have been identified in the genome of the dog: about one nucleotide position in every 900 is variable. The dog genome project resulted in a catalogue of the positions of more than two million SNPs.

[11] A VNTR is a short nucleotide sequence that is organized as a tandem repeat. The length of VNTRs varies between individuals, i.e. multiple alleles exist in the population.

[12] A SINE is a stretch of DNA of less than 500 nucleotides in length. SINES are able to replicate and re-insert themselves into the genome to produce mutations by inserting near or within genes. SINEs represent about 7% of the dog genome sequence (Kirkness *et al*., 2003).

Box 5.1 Genetic architecture of behavior: Lessons from human genetics

Behavior is produced by intricate neural networks that are developed and maintained under influence of a variety of genes and environmental factors (Hamer, 2002; Robinson, 2004). Some of the genes involved in the regulation of behavior may have the same DNA sequence in all individuals, i.e. they are monomorphic. Such genes are important for the regulation of behavior, but they do not contribute to individual *variation* in behavior. Other genes are polymorphic, i.e. different versions of the gene exist in the general population. A genetic polymorphism may affect the structure of the protein encoded by the gene or the level, timing, or tissue-specific expression of certain proteins. Selection – natural or artificial – alters the frequency of genetic polymorphisms and in this way eventually leads to phenotypic changes at the population level.

An illustrative example of a gene involved in the regulation of behavior in humans is the gene encoding monoamine oxidase A (*MAOA*). The MAOA enzyme catalyzes the degradation of the neurotransmitters serotonin, dopamine, and norepinephrine in the brain. These neurotransmitters are involved in the regulation of mood (Fan *et al*., 2010). Brunner *et al*. (1993) described a Dutch family with a point mutation[13] in *MAOA*. Male members of this family produced no MAOA enzyme because the mutation caused a premature stop codon.[14] These males were mentally retarded and showed impulsive violent behavior, including arson, rape, and murder. The researchers hypothesized that accumulation of the neurotransmitters that are normally degraded by the MAOA enzyme lowered the threshold for aggression in these men when under stress (Brunner *et al*., 1993).

The Dutch family had a rare mutation that completely destroyed the function of the *MAOA* gene. The normal range of behavioral variation in humans is the result of many genetic variants with a small effect in combination with environmental influences (Plomin, 1990). In other words, behavior is a complex trait. This is also illustrated by the gene *MAOA*. In the general human population, there are two forms of the *MAOA* gene: a form that is highly expressed and gives rise to high levels of MAOA enzyme, and a low-expressed form. Caspi *et al*. (2002) studied this polymorphism in a large cohort of male subjects that had been followed from birth to adulthood. The aim of the study was to determine why some children who are maltreated grow up to develop antisocial behavior, whereas others do not. Animal studies suggest that maltreatment alters serotonin, dopamine, and norepinephrine neurotransmitter systems in the brain in ways that can influence behavior later in life. Caspi *et al*. (2002) found that childhood maltreatment was a much stronger predictor of adult antisocial behavior in children with a genotype conferring low levels of MAOA expression (Figure 5.2). This suggests that MAOA levels in these children are insufficient to constrain maltreatment-induced changes to neurotransmitter systems. We can conclude that behavior in the general human population is a complex polygenic trait, but that a disruption (e.g. a mutation that causes a premature stop codon) of one of these polygenes can result in a severe deregulation of development.

As in humans, the normal range of behavioral variation in dogs is probably regulated by multiple genetic variants (rare or common) and environmental influences. However, it has been argued that the genetic regulation of complex traits may be simpler in dogs than in humans and that the genetic variants involved have larger effects (Lindblad-Toh *et al*., 2005). For instance, Boyko *et al*. (2010) needed only two to six genetic loci[15] to explain a large

[13] A single DNA nucleotide is altered in a point mutation.

[14] Only males were affected because *MAOA* is located on the X chromosome.

[15] The term "locus" is used to denote a specified position in the genome.

proportion (~70%) of the variation in canine morphological traits such as body size and bone shape. Similar traits in humans are governed by hundreds of loci with small effects (Manolio *et al*., 2009). Boyko *et al*. (2010) explain their finding by the unique population history of the domestic dog, in which novel variants with large effects were preserved by artificial selection for fancy traits, especially during the Victorian era. Lequarré *et al*. (2011) suggest that the small effective population size within many dog breeds has resulted in larger gene effects because these are less effectively counter-selected in smaller populations. It is at this point unclear if this also applies to genes involved in dog behavior, because the theories rely on the assumption that there have been mutations with large effects on the phenotype in the past. Mutations with a large effect on behavior such as the *MAOA* null mutation are very rare (Brunner *et al*., 1993). In addition, behavior was probably never the only parameter that was selected for by dog breeders. Selection for canine behavior may have been more similar to traditional selection for quantitative production traits in livestock. In farm animals, the population averages for economically useful quantitative traits such as milk production, fecundity, and meat quality have been altered by progressive directional selection over many generations. This selection acted on small individual differences in the traits. The genetic architecture of behavior in dogs may, however, be simpler than in livestock because some genetic variants may have been removed from certain breeds by drift or by selection for linked traits. In conclusion, canine behavior is probably controlled by a smaller number of loci than human behavior, but it is still a complex trait involving many genes and gene–gene or gene–environment interactions.

5.3.2 Human behavioral gene mapping

Several reviews of candidate gene studies of human behavioral traits are available in the literature (Burmeister *et al*., 2008, Hill, 2010, Li & Burmeister, 2009). Candidate gene associations have proven difficult to replicate (Hirschhorn *et al*., 2002; Todd, 2006). Possible reasons include inadequate sample sizes leading to spurious associations, differences in the study populations, differences in phenotype definitions, and publication bias (Colhoun *et al*., 2003). This has led to increasing skepticism about the value of candidate gene association studies for detecting genetic variants contributing to complex traits. Meta-analyses are now considered necessary to firmly establish candidate gene associations in human genetics (e.g. Stutzmann *et al*., 2007).

GWAS studies in humans have firmly established associations of genetic variants with a variety of complex diseases (McCarthy *et al*., 2008). GWAS of human behavioral traits have lagged behind those of somatic diseases. In 2011, the catalog of published GWAS of the National Human Genome Research Institute contained 14 papers on schizophrenia, 14 on bipolar disorder, and two on depression (www.genome.gov/GWAStudies/; Liu, 2011). The strongest evidence for a mental disease gene so far comes from an aggregation of GWASs (International Schizophrenia Consortium *et al*., 2009). This study implicated the major histocompatibility complex in schizophrenia. The Psychiatric GWAS Consortium plans to combine psychiatric GWASs to create a data set of 59 000 independent cases and controls and 7700 family trios for use in meta- and mega-analyses (Psychiatric GWAS Consortium Steering Committee, 2009).

5.3.3 Candidate gene association studies of dog behavior

Most candidate gene studies of canine behavior are focused on genes that play a role in personality traits or mental disorders in humans. The candidate genes usually code for aspects of brain neurotransmission systems. An example is the serotonergic system, which plays a key role in the

modulation of behavioral traits in various species (Lesch & Merschdorf, 2000). Serotonergic drugs, for example, are successfully applied in the treatment of impulsive and anxious behavior in humans (Fernandez *et al*., 2001; Messa *et al*., 2003). Biochemical and neuroimaging studies suggest that the serotonergic system modulates behavior in dogs as well (Badino *et al*., 2004; Reisner *et al*., 1996; Vermeire *et al*., 2011; Wright *et al*., 2012).

The majority of studies that have attempted to associate candidate gene polymorphisms with canine behavioral traits focused on aggressive behavior or impulsivity/activity-related behaviors. The behavior of the dogs was measured using owner-report questionnaires, owner reports, or trainer ratings in these studies. Significant associations with measures of aggression were reported for the genes encoding the androgen receptor (Konno *et al*., 2011), the dopamine D1 receptor (Våge *et al*. 2010a), the serotonin receptors 1D and 2C (Våge *et al*., 2010a), solute carrier family 6 (neurotransmitter transporter, gamma-aminobutyric acid) member 1 (Våge *et al*., 2010a), and solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter), member 2 (Takeuchi *et al*., 2009b). The phenotype "impulsivity" is related to inhibitory control (Wright *et al*., 2012). Impulsivity can influence responsiveness to training and the dog's reactions to its environment (Wright *et al*., 2012). "Activity" refers to self-initiated movement (Kubinyi *et al*., 2012). For the activity-impulsivity phenotype, significant associations were reported with polymorphisms in the catechol-O-methyltransferase gene (Takeuchi *et al*., 2009a), the dopamine D4 receptor gene (Hejjas *et al*., 2009), the solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter) member 2 gene (Takeuchi *et al*., 2009a), and the tyrosine hydroxylase gene (Kubinyi *et al*., 2012).

Candidate gene studies illustrate that behavior is not only a product of genes, but also of other factors, including sex and training. An example is the work of Hejjas *et al*. (2007a, 2007b) on the association of polymorphisms in the dopamine D4 receptor gene (*drd4*) with activity-impulsivity in German shepherd dogs. The dopamine D4 receptor is abundant in the limbic system of the brain, a region that is involved in emotion and cognitive function. A polymorphism in the human ortholog of *drd4* may be associated with human personality traits and mental disorders including attention deficit hyperactivity disorder (ADHD) (Benjamin *et al*., 1996; Ebstein *et al*., 1997). A VNTR in exon 3 of *drd4* was studied in German shepherd dogs using a questionnaire measure of activity-impulsivity (Hejjas *et al*., 2007b). The VNTR was significantly associated with the personality measure in police dogs (n = 87), but not in pet dogs (n = 102), suggesting a gene–environment interaction (Hejjas *et al*., 2007b). Environment may include responder variation in this study: there may be a difference in the way those who work with police dogs and those who keep German shepherd dogs as pets answer the questions about dog behavior. Another example is the work of Konno *et al*. (2011), who studied an androgen receptor gene (*ar*) VNTR in Japanese akita inu dogs. Androgens play a key role in modulating behavior. A similar polymorphism in the human androgen receptor gene affects the level of expression of the gene (Chamberlain *et al*., 1994). Questionnaire scores of aggression differed significantly between 30 male dogs with a short version of the VNTR and 24 male dogs with a long repeat. In female dogs, no association between aggression scores and genotype was found, so the association was sex-specific.

The results of the candidate gene association studies described above should be interpreted with caution for several reasons. First, familial relationships between the dogs were not fully taken into account in most studies. This may give rise to spurious associations. Second, candidate gene associations in human genetics have proven difficult to replicate. As was discussed in the section about human behavioral gene mapping, possible reasons include differences in the study populations, differences in phenotype definitions, small sample sizes leading to spurious associations, and publication bias. The same problems can be recognized in the dog candidate gene studies. For

instance, results of association studies of the *drd4* exon 3 VNTR with impulsivity-related phenotypes depended on breed and phenotype definitions. In addition, sample sizes of the dog studies are typically small. It has been suggested that genetic association studies of complex traits require smaller sample sizes in dogs than in humans because of the simpler architecture of the canine genome (see Box 5.1). Simulations estimated that only 100 cases and 100 controls are required to map a complex trait that confers a five-fold increased risk in dogs (Karlsson *et al*., 2007). However, it is still unclear whether this is also true for behavioral traits. Finally, most of the dog studies used a limited number of markers to study the genes of interest. As a result, the studies may not have captured all variation in the candidate gene regions and may thus have missed associations. In conclusion, candidate gene studies of dog behavior are still in their infancy and replication studies are required.

5.3.4 Genome-wide association studies of dog behavior

Canine GWAS became possible with the release of the assembled DNA sequence of the genome of a Boxer named Tasha in 2005 (Lindblad-Toh *et al*., 2005). Tasha's genome revealed the unique architecture of the canine genome. The initial domestication of dogs from wolves, and the later formation of the variety of breeds that exist today, have left indelible marks on the canine genome (Parker *et al*., 2010). The unique structure of the canine genome is expected to facilitate the detection of genetic variants that influence complex traits such as behavior using the dog. Indeed, quantitative trait loci (QTLs[16]) for several breed-defining complex traits have been identified using genome-wide methods in the past few years, e.g. skeletal traits (Parker *et al*., 2009; Sutter *et al*., 2007), hair characteristics (Cadieu *et al*., 2009; Drögemüller *et al*., 2008; Salmon Hillbertz *et al*., 2007), coat color (Candille *et al*., 2007; Karlsson *et al*., 2007), and wrinkled skin (Akey *et al*., 2010). For several of these traits, the causal genetic variant has been identified. An example is the short-legged phenotype of dog breeds such as dachshund, corgi, and basset hound. The short legs seem to be caused by the expression of a retrogene[17] encoding fibroblast growth factor 4 (Parker *et al*., 2009; vonHoldt & Driscoll, Chapter 3).

As in humans, GWAS of canine behavioral traits have lagged behind those of traits related to physical appearance and somatic disease. In 2010, the first GWAS for a behavioral trait in dogs was reported (Dodman *et al*., 2010). The study concerned flank and blanket sucking obsessive-compulsive behavior (OCD) in Doberman pinschers. Canine OCD can manifest as obsessive predatory behaviors (e.g. fly snapping, tail chasing), obsessive oral behaviors (e.g. acral lick dermatitis, flank sucking, blanket sucking) or obsessive locomotion (Brown *et al*., 1987; Dodman *et al*., 1993, 2010; Heywood, 1977; Overall, 2000; Rapoport *et al*., 1992; Schwartz, 1993). OCD is normal behavior manifested in extreme or inappropriate ways (Overall, 2000), and it usually appears first between pre-pubescence and early social maturity. At first, the behavior is triggered by

[16]A quantitative trait is continuously varying in the sense that individuals cannot be readily classified into distinct classes. Many traits of interest in animal breeding, including behavior, are considered quantitative traits. The term "locus" is used to denote a specified position in the genome. A QTL is a stretch of DNA that contains one or more genetic variants that affect a quantitative trait.

[17]A retrogene arises when RNA is copied to DNA and then incorporated into the genome. This process (called reverse transcription) is a common source of novel DNA sequence acquired during the evolution of species. The majority of retrogenes rapidly accumulate mutations that disrupt the reading frame of the gene and thus become inactive. A small percentage become new genes that encode functional proteins.

environmental stressors. Soon it becomes fixed and is displayed in the absence of obvious stressors (Overall & Dunham, 2002). OCD may be improved with treatment, but is generally regarded as incurable and has disastrous consequences for animal welfare. Certain breeds seem predisposed to the disorders. For example, bull terriers frequently exhibit tail chasing, while Doberman pinschers are prone to acral licking. This breed-specific prevalence is suggestive of a genetic origin.

Dodman *et al*. (2010) analyzed 14 700 SNPs in the genomes of around 90 Doberman OCD cases and 70 controls. OCD diagnoses were obtained from veterinarians. They found an association with a SNP on dog chromosome 7. Sixty percent of the Dobermans that showed multiple compulsive behaviors (chewed their flanks, blankets, etc.) had the risk allele, compared with 43% of the dogs with a less severe phenotype and 22% of those without OCD (Dodman *et al*., 2010). The SNP is located in a gene called cadherin 2 (*chd2*). Cadherin 2 is involved in forming connections between neural cells and it is widely expressed. Tiira *et al*. (2011, 2012) subsequently tested the association of the *chd2* SNP with compulsive tail-chasing in bull terriers and German shepherds. They failed to find an association, but these studies may have been underpowered. It is therefore at this point unclear in which breeds and in which types of compulsive behavior the *chd2* gene plays a role. The GWAS study of Dodman *et al*. (2010) found several additional SNPs associated with flank and blanket sucking, suggesting that in addition to *chd2*, other genes contribute to OCD in Dobermans. These may include serotonergic and dopaminergic genes because a neuro-imaging study of Vermeire *et al*. (2012) provided preliminary evidence for imbalances in these neurotransmitter systems in dogs with OCD.

5.3.5 Across-breed genome-wide association studies of dog behavior

An alternative to within-breed GWAS is across-breed genome-wide association mapping (Boyko *et al*., 2010; Chase *et al*., 2009; Karlsson & Lindblad-Toh, 2008). In this method, breed-average phenotypes are used. The breed-average phenotype is associated with the breed allele frequency of a genome-wide set of SNPs that have been genotyped in a large number of breeds. Jones *et al*. (2008) used this method to identify QTLs for several breed stereotypes, including the behavioral traits boldness, pointing, herding, trainability, and excitability. An experienced dog trainer scored the average breed phenotypes for boldness, pointing, herding and trainability as dichotomous variables, e.g. bold or non-bold. Breed phenotypes for excitability were quantitative rates (1–10) derived from the studies of Hart & Miller (1985).[18] Genotypes of 1536 SNPs from 2801 dogs of 147 breeds were used in the study. The majority of breeds were represented by at least 10 dogs. They used 147 (=number of breeds) combinations of breed-specific phenotype (e.g. bold or non-bold) with breed-specific allele frequency of a SNP (=value between 0 and 1) to calculate a Pearson product correlation between phenotype and SNP, weighted for the number of dogs per breed. Twelve loci were identified; five of these contained interesting genes. A herding QTL on canine chromosome 1 contains the genes *mc2r* and *c18orf1*. The former is a melanocortin receptor; the latter has been implicated in schizophrenia. The pointing locus on canine chromosome 8 contains the gene *cnih*, which has been implicated in cranial nerve development. A locus on canine chromosome 22 was identified for boldness. This genomic region contains the *pcdh9* gene, which encodes a protein involved in specific neural connections and signal transduction. Two other loci

[18] Hart & Miller (1985) asked a group of randomly selected veterinarians and dog obedience judges to rank breeds for several behavioral traits (see also Hart & Hart, Chapter 7).

for boldness included the *igf1* gene and the gene encoding the dopamine receptor 1 (*drd1*) (Chase *et al*., 2009). The association of *drd1* and *igf1* with dog behavior is supported by other studies: *drd1* was associated with English Cocker Spaniel aggression in the candidate gene study of Våge *et al*. (2010a). IGF-1 has been implicated in anxiety: nervous pointer dogs have lower serum IGF-1 levels than normal pointers (Uhde *et al*., 1992).

Vaysse *et al*. (2011) also used across-breed mapping to search for genomic regions affecting boldness. They used the same phenotypic definitions as Jones *et al*. (2008), but a different statistical approach in which they assigned each individual dog its breed-specific phenotype and then performed a "normal" GWAS. They examined the dog genome at a higher resolution than Jones *et al*. (2008) because they used more SNPs. When 18 bold and 19 non-bold breeds were compared, a highly significant association was found on chromosome 10, near the gene *hmga2* (high mobility group at-hook 2). This gene is involved in transcriptional activation of genes involved in cell proliferation (Pfannkuche *et al*., 2009). The region was not detected in the study of Jones *et al*. (2008), which is probably due to methodological differences between the studies, e.g. different breeds, different lineages within a breed, different numbers from each breed, and different SNP sets. Vaysse *et al*. (2011) also used DMA data about sociability, curiosity, playfulness, chase-proneness, and aggressiveness for 509 dogs from 46 diverse breeds for across-breed GWAS. A genome-wide significant association for the sociability trait was found on the X chromosome (Vaysse *et al*., 2011). No significant associations were detected for the other DMA traits.

The results of the across-breed studies are very interesting, but they should be treated with caution. The across-breed mapping strategy is sensitive to false positive results due to complex unequal relatedness between breeds (Jones *et al*., 2008). In addition, other complex effects such as interactions between genes or co-selection of loci[19] during breed formation may confound the results (Jones *et al*., 2008). The use of breed-average phenotypes is obviously a simplification of reality. The behavioral phenotypes may vary as much within breeds as across breeds (Figure 5.1). Validation of the loci using within-breed segregation analysis is thus required.

5.3.6 Genome-wide comparisons with other canid species

vonHoldt *et al*. (2010) compared genotypes of more than 48 000 SNPs in 912 dogs and 225 grey wolves. They searched for genomic regions that showed signs of positive selection during early dog domestication (see also vonHoldt & Driscoll, Chapter 3). Two out of three genomic regions that were identified have been implicated in memory formation and/or behavioral sensitization in mice or humans (ryanodine receptor 3 and adenylate cyclase 8). They also found evidence for positive selection for a SNP near the *wbscr17* gene. Mutations in this gene result in Williams-Beuren syndrome in humans, which is characterized by social traits such as exceptional gregariousness. Another genomic region, on dog chromosome 5, is orthologous to a region associated with tame behavior in the farm fox experiment (Kukekova *et al*., 2011a). This makes it plausible that this region is indeed involved in domestication of dogs.

[19] When the frequency of a certain DNA fragment in a breed increases due to selection, the DNA flanking this fragment is also increasing in frequency. This is referred to as co-selection. The flanking regions may contain genetic variants that affect disease risk or behavior. In this way, selection for physical appearance can result in an increased disease allele frequency or in an increase of a genetic variant affecting behavior.

5.4 Discussion

5.4.1 What's in a name: Phenotype

One of the reasons for the slow progress of the field of canine behavioral genetics is the difficulty of measuring behavior (Bearden *et al*., 2004; Mills, 2003; Smoller and Tsuang, 1998). As Felix Brown stated in 1942, "The chief difficulty is to define the condition the heredity of which one is attempting to trace." Early behavioral genetics research in fruit flies and rodents used objective metrics such as timed latencies or frequencies of a behavior. Scott and Fuller also used very specific measures in their classic work, e.g. "barks in a specified time frame." The recent work of Kukekova *et al*. (2011a) also applied objective metrics to fox behavioral genetics. The fox researchers developed a behavioral test in which an investigator approached the caged fox and tried to open the cage and touch the animal. Reactions of the foxes were videotaped and evaluated in a binary manner (yes/no) for a set of traits that involved the fox's body language, actions and position with respect to the investigator. Factor analysis was used to group the behavioral elements into a quantitative measure of tame versus aggressive behavior. These factors were then used as phenotypes for gene mapping (see Section 5.4.4 below).

The dog genetic studies reviewed in this chapter used more subjective phenotypic measures. Most heritability studies used phenotypes based on the behavior of dogs in test batteries. Jones and Gosling (2005) have reviewed studies of canine personality and noted that, "In theory, test batteries were the closest to achieving objectivity, but in practice the levels of objectivity actually attained varied substantially." The molecular genetic studies mostly used even more subjective measures such as owner-report questionnaires and expert ratings (experts being veterinarians, trainers, or dog obedience judges). Owner and expert ratings may be influenced by a variety of factors other than the behavior of the dog, e.g. owner personality and expectations of typical dog behavior. Intuitively, the use of specific and objective metrics in genetic studies seems preferable. However, behavior of dogs in a test battery may not be representative of their behavior in everyday life and it is often unclear what exactly is being measured. Van den Berg and colleagues used three methods for measuring canine aggressive behavior: a behavioral test of the dog (van den Berg *et al*., 2003), a questionnaire for the dog owner (van den Berg *et al*., 2006), and a personal interview with the dog owner (van den Berg *et al*., 2003, 2006). The most promising heritability estimates (i.e. high heritability with low standard errors) were obtained for the owner impressions collected during the personal interview (Liinamo *et al*., 2007). This is rather surprising because of the subjectivity of these phenotypes. Large coordinated projects, such as the European LUPA consortium, make an effort to clarify dog behavioral phenotypes by following standard procedures to describe dog behavior (Lequarré *et al*., 2011). This is of great value for progress in canine behavioral genetics.

5.4.2 Between genes and behavior: Endophenotypes

One potential approach to attack the complexity of behavior is to focus on less complex phenotypic variation, i.e. endophenotypes (Doyle *et al*., 2005). Endophenotypes are closer to the biological basis of a complex trait, e.g. metabolites in urine or blood. Endophenotypes are assumed to be influenced by a subset of the genes that influence the complex trait. This reduced genetic complexity is the result of: (1) the relative proximity of the endophenotype to genes in the chain of events leading

from gene to behavior, and (2) its potential to target one of several pathways that combine to create the trait. The reduced genetic complexity is expected to result in greater statistical power to detect the effects of individual genes. The endophenotype approach is popular in human psychiatric genetics (Robbins *et al*., 2012). Endophenotypes have not been used in canine behavioral genetic studies as yet, but they could include neuroimaging data (e.g. Vermeire *et al*., 2011, 2012), levels of metabolites in blood, urine or saliva (Wright *et al*., 2012), or gene expression data (Våge *et al*., 2010b).

5.4.3 Brain gene expression studies

Gene expression in the brain also occurs in between gene and behavioral phenotype. Large-scale analysis of gene expression has become feasible with massive parallel sequencing technologies. Such techniques sample all transcripts in a tissue, including noncoding sequences (Liu, 2011; Mattick *et al*., 2010). Saetre *et al*. (2004) compared gene expression in brains of wolves and dogs. They suggest that domestication of dogs has resulted in changes in expression patterns of several hypothalamic genes with multiple functions, e.g. the neuropeptides, *calcb* and *npy*. Kukekova *et al*. (2011b) compared the prefrontal cortical brain transcriptome[20] from a tame and an aggressive silver fox. Many of the genes that were overexpressed in the tame fox compared to the aggressive fox were involved in neurological diseases in humans (Kukekova *et al*., 2011b). One of these genes encodes the serotonin receptor 2C (*htr2c*), which has also been reported as being overexpressed in tame compared to aggressive rats (Popova *et al*., 2010). A non-coding SNP in this gene was associated with aggressive behavior in English cocker spaniel dogs in a candidate gene association study of Våge *et al*. (2010a). In the aggressive fox, several genes involved in cardiovascular disease were overexpressed. None of the genes that was differentially expressed in the dog/wolf study of Saetre *et al*. (2004) was differently expressed between the fox samples.

Våge *et al*. (2010b) studied the expression of nine genes in the brains of 11 dogs that were euthanized because of aggressive behavior, and nine non-aggressive dogs euthanized for unrelated reasons. The candidate genes were identified in an initial screening. The studied brain regions were amygdala, frontal cortex, hypothalamus and parietal cortex. These brain areas are involved in emotion. Two of the nine genes, *ube2v2* and zinc finger protein227 (*znf*227) were differentially expressed in brains of aggressive and non-aggressive dogs. The *ube2v2* gene participates in a variety of cellular biochemical processes, including cell proliferation, regulation of DNA repair, regulation of progression through cell cycles, and protein modification. *znf*227 is likely to be a transcriptional regulator, i.e. it affects the expression of various genes. However, the expression differences were very small and formally not statistically significant. Further work, including testing the results in additional individuals, is needed to confirm the results of the gene expression studies discussed here.

5.4.4 Behavior is a complex trait: Epigenetics and epistasis

The slow progress in behavioral genetics is also caused by the fact that behavior is such a complex trait. Gene–environment and gene–gene interactions play a role in behavioral regulation. The study of Caspi *et al*. (2002) is an example of how genes (*MAOA*) interact with the environment (*maltreatment*) to determine biological processes in the brain, and as a result, behavior (*antisocial behavior*) (Figure 5.2). It is becoming increasingly clear that epigenetics may provide an explanation at

[20] The term "transcriptome" is used to denote all transcribed DNA sequence in a certain tissue.

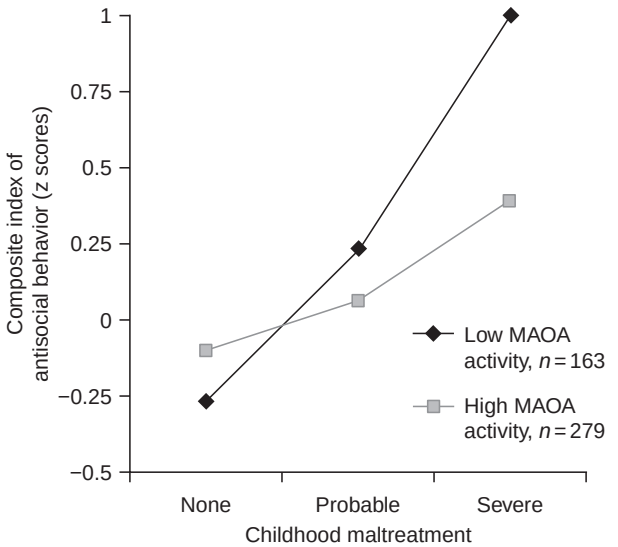

Figure 5.2 Mean antisocial behavior scores as a function of childhood history of maltreatment and MAOA activity in the study of Caspi *et al*. (2002). Subjects were males that were studied from birth to adulthood. Childhood maltreatment is grouped into three categories on the horizontal axis. The vertical axis represents an antisocial behavior composite score, which was standardized to a mean of 0 and SD of 1. MAOA activity (grouped into low and high) is the gene expression level associated with allelic variants of the *MAOA* gene polymorphism. (From Caspi, A. *et al*. (2002). Science, 297, 851–854. Reprinted with permission from AAAS.)

the molecular level for such gene–environment interactions (Hoffmann & Spengler, 2012; Hunter, 2012). Epigenetics refers to mitotically stable changes in gene expression potential that are not caused by changes in DNA sequence. Epigenetic modifications enable the same genotype to produce different cellular phenotypes. Histone modifications and DNA methylation are examples of epigenetic changes. These modifications affect gene expression potential through altered accessibility of the DNA to transcription factors. For instance, dense methylation of CG dinucleotides in a gene promoter results in transcriptional silence. Such changes may even be stable across multiple generations (Stöger, 2008; Waterland *et al*., 2008). Early embryogenesis is a critical period for the establishment of epigenetic patterns (Gluckman *et al*., 2009). For instance, a maternal diet deficient in methyl donors and cofactors results in hypomethylation of the promoter of the agouti gene in mice (Waterland & Jirtle, 2003). The agouti protein induces yellow pigmentation in hair follicles and antagonizes satiety signaling in the hypothalamus. Hypomethylation of the agouti promoter results in yellow coat color and increased prevalence of obesity in the mice offspring. A similar relationship has been observed in humans: prenatal famine exposure during the Dutch Hunger Winter was associated with persistent changes in DNA methylation of genes involved in growth and metabolic disease (Heijmans *et al*., 2008; Tobi *et al*., 2009). No epigenetic studies of dog behavior have been performed yet (although see Koch *et al*., 2016), but it is expected that similar mechanisms are at work.

In addition to gene-environment interactions, gene-gene interactions (termed epistasis) play a role in behavioral variation. Albert *et al*. (2009) showed that a five locus epistatic network influences tameness in rats. An interesting study of Kukekova *et al*. (2011a) suggests that a similar mechanism may be operating in silver foxes. As was described above, the fox researchers developed a behavioral test that resulted in a quantitative measure of tame versus aggressive behavior. The test was applied to several fox populations, including a tame population that was selected for tameness for many generations, an aggressive population that was selected for aggressive behavior for many generations, and their crosses, intercrosses and backcrosses. Mean scores on the tameness factor differed between the populations, showing a linear gradient from tame to aggressive, which is consistent with its heritability (Figure 5.3). The tameness factor mapped to fox chromosome 12, in a region orthologous to the region on dog chromosome 5 that was associated with dog domestication

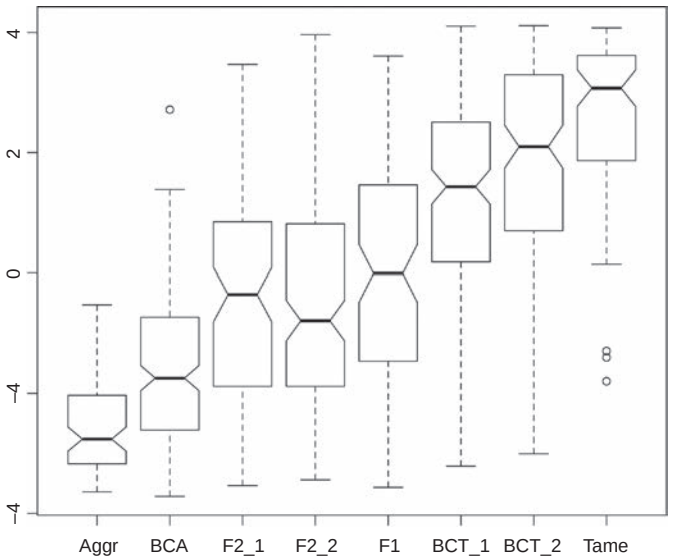

Figure 5.3 Tameness scores of silver fox populations in the study of Kukekova *et al*. (2011a). Tameness scores were derived from a standardized videotaped test in which an investigator approached the caged fox and tried to open the cage and touch the animal. Each fox was evaluated in a binary (yes/no) manner for a set of traits, e.g. wagging the tail, coming to the front of the cage, allowing head to be touched, holding observer's hand with its mouth. Factor analysis was used to detect underlying behavioral patterns in the dataset. The vertical axis in this figure represents scores on a factor that distinguishes between the tame and aggressive fox populations. Aggr = "aggressive" founder population; BCA = backcross-to-aggressive; F2_1 and F2_2 = two different F2 populations (F1 x F1); F1 = F1 population ("tame" x "aggressive"); BCT_1 and BCT_2 = two different backcross-to-tame populations; Tame = "tame" founder population. Horizontal bars within each box represent the population median. Confidence intervals for the medians are shown as notches such that two distributions with non-overlapping notches are significantly different. 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. (Figure reprinted from Kukekova *et al*., 2011a with permission from Springer.)

in the study of vonHoldt *et al*. (2010; see Section 5.3.6). However, the exact region of association within fox chromosome 12 differed between populations, i.e. it depended on genomic context. This suggests that epistasis is involved.

5.4.5 Importance of behavioral genetic studies in the dog

Dogs fulfill important functions in our society as working partners in military and police organizations, as farm dogs, assistance dogs, and guide dogs for the blind. In addition, dogs provide much-valued companionship to humans. Behavioral considerations are important in dog breeding programs because breeding can create better working dogs and better companions. Rapid changes in canine behavioral traits are possible in just a few generations (Svartberg, 2006; Trut *et al*., 2009).

Genomic selection has recently expanded the repertoire of tools available to the breeder to initiate genetic change. Genomic selection calculates a genetic score based on the proportion of favorable genetic markers from a genome-wide set associated with a complex trait of interest. This technique

is already applied in livestock species (Goddard & Hayes, 2009). In dog breeding, the application of genomic selection would require a coordinated effort on a national or international scale (Wilson & Wade, 2012). This might be difficult to achieve because most countries hold many dispersed small dog breeding enterprises. Such small populations, each with different breeding objectives, are probably altered more by genetic drift than by the intended selective pressure applied by the breeder (Bulmer, 1972). Breed registries are also often averse to accepting outcrosses to introduce additional genetic variation to a breed.

Molecular genetic tests could also be used to predict individual risk of problem behavior in the future. This may be very useful in the debate about "breed *versus* deed." Ownership of dog breeds that are considered to be dangerous is restricted in many countries (Overall, 2010; Lockwood, Chapter 9). Breed-specific legislation has also created pressure on animal shelters to employ predictors of aggression risk in dogs. However, the heritability of the behavioral traits presented in this chapter shows that behavior in any individual dog is a product not only of its genes, but also its environment, including access to socialization, training, and exercise (Serpell *et al*., Chapter 6). It is thus uncertain whether a reliable molecular test can be developed, even when complete knowledge of the genetics of canine antisocial behavior would be available. Genetic profiling for personalized medicine has also met criticism in human complex disease genetics (Janssens & van Duijn, 2008). The same is true for gene expression patterns as predictors for problem behavior. Measuring gene expression patterns associated with antisocial behavior may also be difficult to apply in practice due to limited access to the tissues of expression in living animals. A more feasible application of the knowledge of the genes and pathways involved in dog behavior might be the elucidation of drug targets. This may lead to better treatment or prediction of drug efficacy in dogs showing problem behavior.

Studying the genetics of behavior in dogs may also prove important to our scientific understanding of human psychology (Cyranoski, 2010). Evidence for causal associations between specific genetic variants and behavioral traits in humans is often inconclusive (Bearden *et al*., 2004; Colhoun *et al*., 2003; Hamer, 2002; Inoue & Lupski, 2003). Both humans and dogs descend from social species that show cooperative and altruistic behaviors. Many canine behavioral disorders resemble human mental problems that are treated with the same medications (Dodman & Shuster, 1998; Overall, 2000). The genome of purebred dogs has characteristics that are favorable for molecular genetic studies of complex traits (Lindblad-Toh *et al*., 2005). Localizing behavioral genes is thus expected to be substantially easier in dogs than in humans. As soon as causal mutations in canine genes have been found, it will be interesting to study the involvement of the corresponding genes and proteins in human behavior.

This has already proven to be a successful approach for the study of narcolepsy (Chen *et al*., 2009). This disease is characterized by excess daytime sleepiness, striking transitions from wakefulness into rapid eye movement (REM) sleep, and cataplexy triggered by positive emotions. It affects 0.02 to 0.18% of the general human population and is a key to understanding other, more common sleep disorders. Familial cases have been reported, but most cases are sporadic. It is considered a complex trait in humans. Some dog breeds also suffer from narcolepsy. The dogs experience cataplexy during vigorous play or when they are excited by being offered their favorite foods (http://med.stanford.edu/school/Psychiatry/narcolepsy/moviedog.html). As in humans, both sporadic and familial cases are observed. Researchers at Stanford University studied a purpose-bred colony of narcoleptic Dobermans and Labrador retrievers (Lin *et al*., 1999). Linkage analysis and fine-mapping resulted in the identification of mutations in the hypocretin-2-receptor gene (*hcrtr2*) as causal to the canine disease. Subsequent studies in humans showed that the number of hypocretin-containing neurons in post-mortem samples of the hypothalamus of narcoleptic patients was reduced by 90% (Thannickal

et al., 2000). Hypocretin was found to play a role in circadian clock-dependent alertness. The hypocretin system might therefore be a therapeutic target for more common sleep disturbances in humans.

5.5 Conclusion

This review tells us that variation in canine behavior is substantially correlated with underlying genetic variation. Breed differences in behavior exist and behavioral dispositions can be selected for. The relative importance of genetic and environmental influences on dog behavior can be estimated by calculating the heritability. Heritability estimates for canine behavioral traits are mostly low to medium, with an average of 0.20. Researchers have pinpointed some genetic variants involved in dog behavior using candidate gene association studies, within-breed genome-wide association studies, across-breed genome-wide association studies, and by comparisons of the dog genome with those of the wolf and fox. Some of the reported genetic associations seem plausible because they are supported by several studies using different methodologies. However, the majority of results of molecular genetic studies are tentative and require future replication. The identification of genetic variants that affect canine behavior has progressed more slowly than the identification of genetic variants for morphological traits and somatic diseases. Reasons for the slow progress of the field include the difficulty of measuring behavior, and the fact that behavior is a complex trait involving many genes with small effects. Nevertheless, the field of canine behavioral genetics has led to breakthroughs that have informed research on neuropsychiatric disorders in humans. Behavioral considerations are important in dog breeding programs. Programs directed toward changes at the breed level will likely require coordinated national or international efforts.

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