

## Genetics of canine behavior: A review

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**Core tip:** This review incorporates the latest findings in the rapidly moving field of canine behavioral genetics. The genes involved in tameness of foxes and in domestication of dogs from wolves are discussed. The genes involved in several obsessive compulsive behaviors such as flank sucking and circling are mentioned. The genetic and physiological differences between aggressive and non-aggressive dogs of various breeds are emphasized.

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### Abstract

The past decade has seen rapid progress in the field of canid behavioral genetics. The recent advances are summarized in this review. The identification of the genes responsible for tameness in silver foxes is the culmination of a half century of behavioral testing and, more recently, genomic investigation. There is agreement that domestic dogs evolved from wolves, but when and from which population remains controversial. The genetic differences between wolves and dogs identified include those for neurotransmitters and digestion. Breed differences in behavior are well known, but only recently have the genetics underlying these differences been investigated. The genes responsible for flank sucking in Doberman Pinschers and for several other obsessive compulsive problems in other breeds have been identified. Aggression is the least desirable canine trait, and several laboratories have detected differences in neurotransmitters and their receptors between aggressive and non-aggressive dogs. In English Cocker Spaniels, the genes linked to aggressive behavior code for dopamine, serotonin, and glutamate receptors. A dopamine transporter gene has been associated with impulsive behavior in Malinois.

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### INTRODUCTION

This review is a summary of recent research focusing on the current knowledge of the genetic contribution to behavior in the Canidae family. We first review the farm fox experiment and how this long-term study has led to greater understanding of the process of canine domestication at the phenotypic and molecular levels. We then turn our attention to the relationship between dogs and wolves and canine breed differences in behavior. Finally, we review the current knowledge of the genetic basis of aggressive behavior in dogs.

### TAME FOX EXPERIMENT

The farm-fox experiment constitutes a major milestone in canid behavioral genetics, clearly demonstrating the genetic basis of behavior. No discussion of the genetics of canine behavior would be complete without summarizing some of the groundbreaking research performed at the Institute of Cytology and Genetics (ICG) of the Russian Academy of Sciences<sup>[1-3]</sup>. For more than 50 years,

scientists at the ICG in Novosibirsk, Russia have been reconstructing experimentally the domestication process in farm-bred silver foxes (a variant form of the red fox, *Vulpes vulpes*) as a novel model for studying the genetic basis of canine domestication and behavior. In 1959, scientist Dmitry Belyaev and his team began an intensive selective breeding program of silver farm foxes to isolate the trait of tame behavior towards humans<sup>[2,4]</sup>. After several generations of selective breeding in a controlled environment, Belyaev succeeded in attaining a subset of tame foxes. During fifty years of continuous selective breeding, the farm-fox experiment has tested over 52000 foxes for tameness, with the resultant tame population of foxes showing friendly dog-like responses to humans as early as one month of age<sup>[2,3,5]</sup>.

The goal of selective breeding of the farm foxes was limited strictly to behavioral criteria related to tameness. However, physical, developmental, physiological, and other behavioral differences also emerged in the tame foxes compared to the original farm-bred foxes. The selection for tameness led to numerous physical changes in the foxes, including piebald coats, floppy ears, and curly tails, despite no selection criteria for these traits<sup>[2]</sup>. In addition, the socialization period elongated from approximately 45 to 60 d in the selected tame foxes, similar to the socialization period in the domestic dog<sup>[1]</sup>. Tame foxes also developed a novel repertoire of affiliative vocalizations towards humans to promote interaction<sup>[6]</sup>. Hare *et al.*<sup>[7]</sup> found that tame fox kits are as skillful as puppies in using human point and gaze gestures for finding hidden food, demonstrating that domestication has led to improved social cognitive ability. Physiological differences also were found with hormonal assays showing that tame foxes do not experience stress when in contact with humans. A comparative study of hypothalamic-pituitary-adrenal axis (HPA) function in tame *vs* unselected foxes showed that in tame foxes, basal and stress-induced blood cortisol levels were respectively three- and five-fold lower than in the unselected foxes<sup>[2,8]</sup>.

In the 1970s, a second parallel strain of farm foxes began to be bred selectively at the Institute for Cytology and Genetics—those with aggressive behaviors towards humans. Fifty farm-bred silver foxes with the most aggressive responses towards humans were selected and used as the basis of the aggressive population<sup>[9]</sup>. Criteria for measuring behavior in the aggressive population were the critical distances between the experimenter and the caged animals at which the animals first demonstrated aggression and the intensity of the aggressive responses<sup>[10]</sup>.

From the evolution of these tame and aggressive populations of foxes, much information has been learned about the changes that can occur with intensive behavior selection pressures. Because the fox-farm domesticated foxes were created in only a few decades through intense selection and by focusing exclusively on certain behavioral traits, it seemed reasonable to assume that a small number of genetic loci determined the behavioral traits<sup>[11]</sup>. A rudimentary map of the fox genome with karyotype

and some linkage groups was available by the late 1990s; however, a meiotic linkage map of the fox was needed to determine which loci were implicated in tame behavior<sup>[9]</sup>. Fortunately, the fox and the dog share a close evolutionary and genetic relationship, and since the dog genome was sequenced by 2005<sup>[12]</sup>, available canine genomic information then could be utilized to develop the necessary fox meiotic map<sup>[3,11]</sup>. The availability of high resolution canine genome maps and sequence data aided in the creation of the fox meiotic linkage map, with the high genomic sequence identity between dog and fox permitting the adaptation of canine microsatellites for genotyping and meiotic mapping in foxes. Using 320 such markers, Kukekova *et al.*<sup>[3]</sup> constructed the first meiotic linkage map of the fox genome. This first mapping covers 16 fox autosomes and the X chromosome. After alignment with a canine genome sequence of similar length, high conservation of marker order between homologous regions of the two species was apparent<sup>[11]</sup>. Utilizing and adapting scoring systems (for tameness and aggression phenotypes) developed by the fox-farm experiment over the years for the selective breeding process, Kukekova *et al.*<sup>[3]</sup> created a new principal-component analysis of fox behavior with selected traits. This new scoring system effectively reduced 311 binary scoring behaviors to fifty of the most important traits that would serve as quantitative phenotypes (and continuous variables) to represent heritable differences in behavior among individual foxes and the fox populations and permit quantitative genetic analysis<sup>[10]</sup>. By interval mapping using fox and canine meiotic maps, a locus for tame behavior on fox chromosome VVU12 was identified. This locus is orthologous to a genomic region implicated in canine domestication<sup>[13]</sup>. Tameness as the defining trait of domestication is a complex “phenotype” consisting of many behavioral variables. In fact, when genome-wide association studies were performed by Kukekova *et al.*<sup>[3]</sup>, the resulting data suggested that at least two VVU12 loci are associated with tame *vs* aggressive behavior and active *vs* passive behavior. Moreover, differing mapping characteristics of specific behavioral traits were found, suggesting different genotype/phenotype relationships; for example, floppy *vs* erect ears are associated with different regions of VVU12 and vary between tame and aggressive foxes. Expression of the VVU12 loci thus appears to depend on interaction with other parts of the genome and on individual fox parents<sup>[13]</sup>.

At the molecular level, the development of transcriptome sequencing significantly enhances genetic study without the need for a fully sequenced genome. The comparison of transcriptome sequencing from the prefrontal cortices of a tame and an aggressive fox is in the preliminary stages at this time<sup>[9]</sup>. Thus far, preliminary analysis of “comparison of transcriptome sequences of the same genes between the tame and aggressive fox samples has identified a large set of informative single nucleotide polymorphism (SNP) markers and begun a catalogue of gene-specific sequence variants between the

two strains<sup>113]</sup>.

The farm-fox experiment demonstrates that over generations, intensive selection for tame behavior in foxes can serve as a reliable model for studying the genetic basis of canine domestication. The identification of genetic loci that both influence tame behavior in foxes and are homologous to regions in the dog genome supports the hypothesis that domesticated behavior in dogs and foxes may have similar genetic bases. These recent advances will help identify more genes implicated in fox behavior that can be correlated to dog domestication.

## WOLVES TO DOGS

The complete sequencing of the dog genome has greatly expanded general knowledge of the processes of genome evolution and the genetic basis of phenotypic traits in dogs and other animals. However, the evolutionary path leading from wild ancestor to domesticated dog continues to remain elusive. Comparative genomics utilizing the completed dog genome has confirmed the close relationship of dogs to such other canidae as foxes, coyotes, and wolves. It appears that modern canids share a common ancestor dating back approximately ten million years; the closest relatives to the dog such as the gray wolf and coyote share a common ancestor dating to approximately three to four million years ago<sup>114]</sup>. Like the dog, all wolf-like canids have 78 chromosomes and can mate with one another to produce fertile offspring. Thus, wolf-like canid species are among the strongest candidates for the ancestors of today's dog. Moreover, molecular genetic data from the past two decades<sup>112,115,116]</sup> strongly support the origin of the dog from the gray wolf in particular<sup>117]</sup>. Molecular evidence also suggests that divergence of dog from wolf and the beginning of the dog's relationship with humans occurred as recently as 15000 years ago<sup>114]</sup>. Other studies looking at genomic variation in wolves, Chinese indigenous dogs, and modern breeds point to an even earlier beginning to domestication, possibly about 30000 years ago, prior to the development of an agricultural human society<sup>118,119]</sup>. Very early domestication may have involved the intentional taming of small groups of wolves who, less fearful of humans and motivated by hunger, scavenged the camps of Mesolithic human hunters-gatherers<sup>120]</sup>.

Where canine domestication originated also is debatable. While DNA genomic data suggest a Middle Eastern origin, analyses of mitochondrial DNA and Y-chromosome markers from various dog breeds and from geographically-dispersed wolf populations suggest that canine domestication originated in East Asia<sup>11]</sup>. Wang *et al.*<sup>118]</sup> used whole-genome sequencing to compare gray wolves, Chinese indigenous dogs, and modern breeds. They found that the genetic variation between the three canid groups generally decreased step-wise from wolf to Chinese dog to modern dog breed. Based on these findings, they speculate that the Chinese indigenous dog may represent the link between wolf and dog and the pro-

genitor of today's diverse modern dog breeds. They identified 311 genes that appear to have been selected in dogs compared to wolves and that have functions affecting sexual reproduction, digestion/metabolism, neurological processes, and cancer. The fact that these particular genes overlap to a great extent with those also selected in humans suggests a parallel evolutionary process in dogs and humans, especially in the realm of neurological processes. They note that: As domestication is often associated with large increases in population density and crowded living conditions, these "unfavourable" environments might be the selective pressure that drove the rewiring of both species. Positive selection in neurological pathways, in particular the serotonin system, could be associated with constant need for reduced aggression stemming from the crowded living environment<sup>118]</sup>.

Another study employed mitochondrial DNA sequencing, showing a closer relationship of dogs to gray wolves from East Asia<sup>121]</sup>. VonHoldt *et al.*<sup>122]</sup> sought to identify the primary source of genetic diversity for domestic dogs and conducted an extensive genome-wide survey of over 48000 SNPs in dogs and gray wolves. Their data, however, showed that dogs share a greater percentage of multi-locus haplotypes unique to gray wolves from the Middle East rather than from East Asia<sup>122]</sup>.

Although genetic data support the theory that the process of canine domestication began in East Asia over 15000 years ago, a recent study compared the complete mitochondrial genome sequences of 18 European prehistoric canids to a comprehensive panel of modern dogs and wolves. The researchers found phylogenetic relatedness between the modern dogs and the ancient canids of Europe dating back to more than 30000 years ago, thus suggesting that canine domestication first may have occurred in Europe rather than in Asia<sup>123]</sup>.

Behavior differences between dogs and wolves are the most striking result of the domestication process, even more than the marked differences in physical size and shape. In fact, the canine breeds in existence today have diverse physical characteristics that distinguish them from one another just as much as from wolves. However, the fact that all breeds of domestic dog as a group are more similar in behavior when compared to one another than when compared to the wolf suggests that genetic selection for behavior drove the domestication process. It is logical to hypothesize that ancestral wolves initially may have experienced natural selection for tame behavior, permitting coexistence with humans. Based on the findings of the fox-farm experiment where genetic loci influencing tame behavior in foxes are homologous to regions in the dog genome and also related to selection differences between dogs and wolves, it is plausible to suggest that domesticated behavior in dogs and foxes share a similar genomic basis<sup>11]</sup>.

Several studies before and after the complete sequencing of the dog genome in 2005 have attempted to target, at the molecular level, the genetic basis of behavioral differences between the domestic dog and its wolf progeni-

tor. A study by Sætre *et al.*<sup>[24]</sup> used microarray technology to evaluate mRNA expression levels of 7762 genes in the post-mortem brains of dogs, wolves, and coyotes. They found markedly altered gene expression of two neuropeptides, CALCB and NPY, in the dogs as compared to the wolves and coyotes. These neuropeptides, present in all mammalian brains, are implicated in energy control and feeding behavior, neuroendocrine stress response *via* the HPA axis, and possibly play a role in anxiety and depression. The findings of species-specific differences in the elaboration of the neuropeptides suggest that selection for behavior during domestication may have resulted in modification of mRNA expression patterns in genes located in the hypothalamus of the dog<sup>[24]</sup>. Björnerfeldt *et al.*<sup>[25]</sup> postulate that domestication of dogs created a new lifestyle that changed selective forces acting on the species, in turn affecting the dog's genome. Using mitochondrial DNA sequencing in 14 dogs, 6 wolves, and 3 coyotes, they showed that dogs have accumulated into their genome non-synonymous changes in mitochondrial genes at rates faster than in wolves. In turn, this results in elevated levels of protein variations in the dog as compared to the wolf. Björnerfeldt *et al.*<sup>[25]</sup> conclude that an important consequence of domestication is a "relaxation of selective constraint on dog mitochondrial DNA" that also could have affected other parts of the dog genome to facilitate "the generation of novel functional genetic diversity"<sup>[25]</sup>. Cruz *et al.*<sup>[26]</sup> compared the genome of the dog to that of the gray wolf to examine the effect of domestication. Using whole-genome SNP data, they compared the variation in dog and wolf genes. They also found increased frequency in the trend for non-synonymous mutations in dogs as compared to their wild canid counterparts. They concluded that the increase in mutation rate could have myriad effects, some deleterious, and may indicate that the process of domestication in the dog led to an increase in functional genetic variation that has contributed to the markedly diverse physical and behavioral phenotypes characteristic of dog breeds, as well as to the prevalence of pathology in modern breeds<sup>[26]</sup>.

Li *et al.*<sup>[27]</sup> studied the expression profiles of a specific subset of developmental genes believed to be implicated in the evolution of dog domestication. They ran comparative genomic analyses by assaying the SNP genotypes in Chinese native dogs (believed to have the genetic structure most similar to that of ancient dog), German Shepherd (purebred) dogs, and gray wolves to detect a genetic basis for the behavior transformation from wolf to primitive dog to modern purebred dog<sup>[27]</sup>. Genomic regions that have undergone strong selection in the recent past should show extended haplotype homozygosity<sup>[28]</sup>. Following this line of reasoning, Li *et al.*<sup>[27]</sup> detected four regions of high extended haplotype homozygosity that contained only a single highly differentiated SNP located within a single gene. Comparison of candidate genes between the Chinese native dogs and wolves showed a high bias for expression localized in the brain's prefrontal cortex, the center for complex cognitive-type behaviors.

However, candidate genes showing large population differentiation between the Chinese dogs and German Shepherds did not demonstrate significant expression bias. Thus, the finding that wolves and dogs have highly differentiated brain-based genes suggests that behavioral transformation most likely was key to the onset of domestication and that "this rapid evolution likely was driven by artificial selection during the primary transition from wolves to ancient dogs, and was consistent with the evolution of dog-specific characteristics, such as behavior transformation, for thousands of years"<sup>[27,28]</sup>.

Other recent studies have taken a closer look at the genetic processes underlying physiological and behavior differences resulting from dog domestication. Utilizing whole-genome resequencing of wolves and dogs, Axelson *et al.*<sup>[29]</sup> identified 36 genomic regions that likely are implicated in selection during the domestication of the dog. It is of interest that more than half of the regions play roles in brain function with 8 regions in particular involved in neurophysiologic pathways that may underlie behavioral changes characteristic of dog domestication. Moreover, they identified 10 genes with selection signals that play key roles in starch digestion and fat metabolism. In terms of starch digestion, three genes (*AMY2B*, *MGAM*, and *SGLT1*) that facilitate the digestion of starches show evidence of being selected for during the process of dog domestication. These findings may indicate that, unlike in carnivorous wolves, genetic mutations found in modern dog facilitate the adaptation to and even thriving on a diet available in cohabitation with humans<sup>[29]</sup>.

## BREED DIFFERENCES

Over the past hundreds of years, the selective breeding of domestic dogs has given rise to more than 400 modern dog breeds with many unique differences in both physical appearance and behavior characteristics<sup>[30]</sup>. The physical differences among the dog breeds mostly are obvious to the naked eye, and the behavior differences between breeds also are distinctive and diverse<sup>[31]</sup>. Humans have exerted genetic pressure on dogs by selecting various traits to create breeds better adapted to utilitarian purposes such as herding, guarding, or hunting. The modern dog's extraordinary diversity in phenotype, behavior, and ability to perform tasks is unmatched by any other species on earth<sup>[32]</sup>. A study by McGreevy *et al.*<sup>[33]</sup> investigated the relationship between height, bodyweight, and canine cephalic index (CI: the ratio of skull width to skull length) and how these values correlated with certain behavior traits using the Canine Behavioral Assessment and Research Questionnaire (C-BARQ). It is of interest that certain canine morphotypes were associated reliably with particular behavior profiles. For example, brachycephalic skull shape (high CI) may be a by-product for human selection of neotenuous behavioral characteristics, and dolichocephalic skull shape is a product of human selection for hunting and chasing ability. The authors note that it is unclear if these associations between mor-



phology and behavior represent functional co-adaptations or accidental by-products of allometric change. Therefore, the relationships noted in this study could be either genetically or environmentally driven or both<sup>[33]</sup>.

With its wealth of phenotypic diversity, the dog clearly is a valuable genetic model for studying both breed-specific behaviors and abnormal behaviors. The persistence of such breed-specific behaviors as herding, pointing, tracking, and hunting in the absence of training or motivation suggests that these behaviors are, at least in part, controlled at a genetic level<sup>[34,35]</sup>.

Prior to the completion of the dog genome in 2005, genetic studies used mitochondrial sequencing to reveal a large amount of variation in relatively short sequences. Although some breed clustering could be demonstrated, researchers found that mitochondrial sequences were more successful at distinguishing between species than between breeds<sup>[36]</sup>. Early genetic studies also utilized microsatellite-based marker sets to study the genomes of a small number of breeds. Differences in allele frequencies occurred in different breeds supporting the hypothesis that there was less variation within breeds than across the species<sup>[36]</sup>. Parker *et al.*<sup>[37]</sup> investigated the relationships among 85 breeds using 96 microsatellite markers, demonstrating marked population stratification within the dog species and establishing that the breeds were indeed genetically separate. Once the whole genome of the dog became available, use of SNPs became favored over microsatellites due to the ease of genotyping bialleles and analyzing thousands of markers in a single assay<sup>[36]</sup>. SNP genotyping chips were derived from the over 2 million SNPs in the dog genome<sup>[38]</sup>. Both analytic techniques are useful; clustering analysis using mitochondrial DNA demonstrates hybridization among groups, while SNP analysis results in a phylogenetic tree that show the unique placement of a breed within a group<sup>[36]</sup>. Moreover, SNP analysis corroborates earlier research showing that genetic variation among breeds is greater than that among individuals. A study by Vonholdt *et al.*<sup>[22]</sup> demonstrated a 4% overall variation between breed clusters.

In the 1950s through the 1960s, Scott and Fuller pioneered research on identifying heritable differences in behavior and cognition in the dog using five different breeds in a laboratory model setting<sup>[39]</sup>. More recent studies have assessed heritability of behavior in working and/or pet dog populations outside of the laboratory setting<sup>[40]</sup>.

In 1989, the Swedish Dog Mentality Assessment (DMA) was initiated as a tool for selective breeding in working dogs. The test originally was developed as a tool for selective breeding of working dogs, but it is used today as a general behavioral test by many breeding clubs in Sweden. The DMA has been applied to over 24000 dogs representing more than 180 breeds. Using this data set and the pedigrees of German Shepherds and Rottweiler dogs, Sætre *et al.*<sup>[41]</sup> noted that the genetic correlation of the score on one test was not independent of the score on another test. In fact, their analysis provides evidence that

there may be substantial shared genetics underlying most of the behavioral response in all of the test situations except for aggression which tended to be distinct. Sætre *et al.*<sup>[41]</sup> identified “shyness-boldness” as a generalized trait underlying many behavioral scores with a heritability of 0.25-0.27.

Recent research has shown that the genetic similarities among different breeds may not correlate well to characteristic behavior traits attributed to historical functional breed groups such as herders and hunters<sup>[30,42]</sup>. Turcsan *et al.*<sup>[30]</sup> investigated whether or not behavioral traits historically believed to characterize certain breed categories actually correlated with genetic relatedness. Using online questionnaires submitted by 5733 dog owners of 98 breeds, they looked at trainability, boldness, calmness, and dog sociability. They found that the breeds differed to a great extent in the four traits and that breed-specific behavior in trainability and boldness appeared to be determined partly by genetics. However, breeds that were similar in behavioral characteristics per report of the owners did not correspond well to recognized functional/conventional breed classification nor to genetic breed clusters. The authors state that this lack of correlation between the questionnaire results and commonly acknowledged breed or functional group traits could be associated with cross-breeding with breeds of dissimilar behavioral traits or could represent differences in socialization and/or relationship with owners. The authors conclude “...the behavioural breed clusters showed poor correspondence to both the functional and genetic categorization, which may reflect the effect of recent selective processes. Behavioural breed clusters can provide a more reliable characterization of the breeds’ current typical behaviour”<sup>[30]</sup>.

Meyer *et al.*<sup>[43]</sup> estimated the heritability and correlation of 7 behavioral traits in German Shepherd Dogs in Switzerland using data from 4855 animals that underwent the standardized behavior test of the German Shepherd Dog Club of Switzerland between 1978 and 2010. The traits tested were self-confidence, nerve stability, hardness, temperament, sharpness, defense drive, and reaction to gunfire. Sex, year of testing, judge, place of testing, and age at testing were found to have significant effects on the outcome of the test. Overall, estimated heritability of the traits was low, ranging from 0.05 (5%) to 0.21 (21%). It also is of interest that some traits were highly correlated; self-confidence and nerve stability had a genetic correlation of 0.98 and sharpness and defensive drive, 0.93. Meyer *et al.*<sup>[43]</sup> suggest that while the heritability of behavioral traits is generally low, genetic evaluation of behavior can be helpful as a basis for selection of a given trait, with the caveat that precise definition of the desired traits along with accurate scoring of the dog’s behavior are requisite<sup>[43]</sup>. Mehrkam *et al.*<sup>[44]</sup> recently reviewed the current state of knowledge regarding canine breed differences in behavior, finding scientific evidence for differences both between breeds as well as within-breed differences<sup>[44]</sup>.

The genetics underlying racing performance has been

studied in sled dogs<sup>[45]</sup>. The Alaskan sled dog is considered genetically distinct in that the population has been shaped to create a group of high-performance athletes through selective interbreeding with purebred dogs based on working ability rather than breed physical appearance. New breeds have been introduced gradually into the lines of racing dogs to improve racing performance. Therefore, Alaskan sled dogs provide a unique opportunity to research the impact of trait selection and breed composition and their influence on genomic structure. Huson *et al*<sup>[45]</sup> genotyped 199 Alaskan sled dogs using 96 microsatellite markers and compared the data to that from 141 genotyped purebred breeds. The breed composition of each sled dog was compared to its performance phenotype, including speed, endurance, and work ethic. It is of interest that the sled dogs separated into two groups that aligned with their racing style-sprint *vs* distance<sup>[46]</sup>. Huson *et al*<sup>[46]</sup> then used a set of 7644 ancestry informative marker SNPs to model ancestry in the sprint and distance sled dog populations with four known reference breeds, the Alaskan Malamute, Siberian Husky, German Shorthaired Pointer, and Borzoi. It was found that the distance sled dogs had, on average, highest Alaskan Malamute allele patterns compared to the sprint dogs who had the highest German Shorthaired Pointer allele patterns. In addition, genetic comparison between sprint *vs* distance racing Alaskan sled dogs identified several genomic regions associated with differences in racing style and pinpointed a variant of *MYH9* gene that is associated with increased heat tolerance in sprint dogs<sup>[46]</sup>. Although variants responsible for improved muscle function are important, those responsible for the motivation to perform are also involved.

There are many genetic differences in behavior, but few of the genes are known. The laboratory of Veterinary Ethology of Tokyo University has located putative genes affecting canine behavior. The researchers have identified polymorphisms in five breeds of dogs (Golden Retriever, Labrador Retriever, Maltese, Miniature Schnauzer, and Shiba) that pinpoint differences in SNPs in genes regulating neurotransmitters, the enzymes that synthesize or destroy the neurotransmitters, and the receptors<sup>[47]</sup>. SNP (T199C) is located on the putative third exon of the canine monoamine oxidase B gene that causes an amino acid substitution from cysteine to arginine. Takeuchi *et al*<sup>[47]</sup> also found 4 SNPs in the tyrosine hydroxylase and dopamine beta hydroxylase genes. Ogata *et al*<sup>[48]</sup> found 2 SNPs in the glutamine transporter gene. The Tokyo University researchers have related the polymorphisms with breed behaviors as identified by Hart *et al*<sup>[49]</sup>, although there is no direct evidence that these could explain interbreed differences<sup>[51]</sup>.

Due to the great diversity of dog breeds, the dog is a valuable genetic model for studying both breed-specific behaviors and abnormal behaviors. At a molecular level, analytic techniques to study breed differences include using mitochondrial DNA to perform cluster analysis that shows hybridization among groups and SNP analysis that develops a phylogenetic tree and places a breed

within a group on that tree. Recent studies also have assessed heritability of behavior in both working dog and pet dog populations. The genetic similarities among different breeds may not correlate well to characteristic behavior traits attributed to historical function of the breed groups. However, behavioral breed clusters may provide a more reliable characterization of the breeds' current typical behavior. Currently, only a few genes that underlie inheritable behavior characteristics are known. Polymorphisms have been identified in five breeds of dogs, pinpointing differences in SNPs in genes regulating neurotransmitters, enzymes acting on neurotransmitter enzymes, and receptors.

## GENETICS OF ABNORMAL BEHAVIOR

### Flank sucking

Yokoyama *et al*<sup>[50]</sup> pointed out that genome wide association testing is more profitable than the candidate gene approach to determining the genetics of behavior. Using this approach, the first gene for a specific behavior was found. Flank sucking, a very specific and easily recognized compulsive problem, is a behavior seen almost exclusively in Doberman Pinschers. *CDH2* is the gene associated with this compulsive behavior. Occasionally a blanket or another material can serve as the substrate for sucking. It is not a serious behavior problem because the irritation to the skin is mild. More owners complain about fabric sucking because the material must be replaced. The sucking behavior occurs mostly as the dog is resting prior to sleeping. Using genome-wide analysis, Dodman *et al*<sup>[51]</sup> found an association of SNPs peak on canine chromosome 7. The most significantly associated SNP is located within the *CDH2* gene. *CDH2* is widely expressed, mediating synaptic activity-regulated neuronal adhesion. Dogs showing multiple compulsive behaviors have a higher frequency of the risk allele than do dogs with a less severe phenotype (60% and 43%, respectively) compared with 22% in unaffected dogs<sup>[51]</sup>.

In an interesting follow-up to the genetic basis of this abnormal behavior, Ogata *et al*<sup>[52]</sup> found that the brains of flank sucking Dobermans differed from those of unaffected Dobermans. Magnetic resonance imaging revealed higher total brain and gray matter volumes and lower dorsal anterior cingulate cortex and right anterior insula gray matter densities in the affected dogs. The affected Dobermans also had higher fractional anisotropy in the splenium of the corpus callosum, the degree of which correlated with the severity of the behavioral phenotype<sup>[52]</sup>.

Another behavior abnormality, tail chasing, can have multiple etiologies including neuropathic pain, so it is not surprising that there is no association with the *CDH2* gene<sup>[53,54]</sup>. Single photon emission computed tomography (SPECT) was used with <sup>123</sup>I-R91150 and <sup>123</sup>I-FP-CIT, in combination with <sup>99m</sup>Tc-ECD brain perfusion co-registration, to measure the serotonin (5-HT) 2A receptor, dopamine transporter (DAT), and serotonin transporter (SERT) availability. There was significantly less 5-HT2A

receptor binding in the frontal and temporal cortex of obsessive compulsive dogs. The midbrain SERT also was lower. The DAT differences between normal and compulsive dogs were mixed<sup>[53]</sup>.

More recently the original data from Dodman *et al.*<sup>[51]</sup> was reanalyzed using a new calling algorithm called MAGIC was used to identify genes, in addition to cadherin, that are involved in flank sucking and other obsessive compulsive behavior (OCD). The genome wide association revealed 119 variants in evolutionarily conserved sites that are specific to dogs with OCD. Using small numbers of dogs, (< 16 of each breed), case dogs (exhibiting OCDs), control dogs, and unphenotyped dogs were compared. Four genes have an excess of case-only variation in evolutionarily constrained elements, even after correcting for gene size: ataxin-1 (ATXN1), neuronal cadherin (CDH2), catenin alpha2 (CTNNA2), and plasma glutamate carboxypeptidase (PGCP). CDH2, a neural cadherin, encodes a calcium dependent cell-cell adhesion glycoprotein important for synapse assembly, where it mediates presynaptic to postsynaptic adhesions.

CTNNA2 encodes a neuronal-specific catenin protein that links cadherins to the cytoskeleton. ATXN1 encodes a chromatin binding protein that regulates the Notch pathway<sup>[42]</sup>, a developmental pathway also active in the adult brain, where it mediates neuronal migration, morphology and synaptic plasticity<sup>[55]</sup>. All three of these genes are involved in synaptic formation. The fourth gene PGCP, encodes a poorly characterized plasma glutamate carboxypeptidase. It may be involved in the hydrolysis of N-acetylaspartylglutamate. One might consider glutamate targeting drugs for treatment of OCD's.

## NEUROTRANSMITTERS AND AGGRESSION

Canine aggression has been the subject of many genetics studies because it is the most common behavior presented as a problem and the only one responsible for human injury or even death<sup>[56]</sup>. Hyperactivity and impulsive (unpredictable) aggression by dogs are problems frequently presented to veterinarians. Since behavior is the consequence of central nervous activity, it is not surprising that differences in neurotransmitters are associated with differences in behavior. These differences can be at any stage in the production and function of the neurotransmitter. The levels of neurotransmitter or their metabolites in brain, blood or cerebral spinal fluid have been investigated, and transporters and receptors of neurotransmitters have been associated genetically with aggression and other behaviors.

Dopamine and serotonin are the neurotransmitters examined most frequently in studies of aggression. Serotonin is produced from tryptophan and is widely believed to be important in the etiology and treatment of mood disorders, including aggression in dogs<sup>[57]</sup>. It is logical to conclude that serotonin levels in the body fluids or number of serotonin receptors should be measured in normal

and abnormal dogs with the prediction that serotonin levels would be lower in aggressive dogs. The results of these studies are summarized below.

Dopamine (D1 and D2) is formed from tyrosine and catalyzed by the enzyme tyrosine kinase. Dopamine has multiple receptors and is inactivated by another enzyme, monoamine oxidase (MAO). Dopamine is transported back into the pre-synaptic neuron *via* a transporter. Studies in dogs exhibiting aggression have examined blood and cerebrospinal fluid levels of dopamine and its expression in the brain. In genetic studies, alleles regulating dopamine transporters, receptors, and dopamine deactivating enzymes have been compared in non-aggressive dogs and dogs exhibiting aggression. The results of these studies are summarized below.

### Blood and body fluids

Cakiroğlu *et al.*<sup>[58]</sup> found that serotonin in blood varied with canine disposition. Serum serotonin was 33 ng/mL in non-aggressive dogs and 12 ng/mL in aggressive dogs<sup>[58]</sup>. In a later study, Leon *et al.*<sup>[59]</sup> found lower levels of serotonin in plasma, serum and platelets in aggressive dogs of various breeds that presented to a behavior clinic than in the control group of Beagles. However, the differing serotonin levels might represent breed differences in serotonin rather than differences between aggressive and non-aggressive dogs.

It is probably more fruitful to look for genetic differences between dogs within the same breed. For that reason, English Cocker Spaniels were studied because dogs of that breed frequently exhibit unpredictable or impulsive aggression towards their owners<sup>[60]</sup>. Moreover, the prevalence of aggression varies with coat color; red (blonde or buff) spaniels are more aggressive than black ones and solid color spaniels are more likely to be aggressive than parti-colored ones. It is not clear how the production of pheomelanin (yellow pigment) rather than melanin (black pigment) leads to or is related to aggression although melanin and dopamine share a common precursor-tyrosine. This area bears investigation<sup>[61]</sup>. Amat *et al.*<sup>[62]</sup> compared serum serotonin levels in aggressive English Cocker Spaniels with those of aggressive dogs of a variety of other breeds and found the serotonin levels were significantly lower in the cockers.

MAO-A is an enzyme that catalyzes monoaminergic neurotransmitters such as dopamine and serotonin. A mutation that lowers the amount of MAO-A is associated with incarcerated humans, if they had bad childhood environments<sup>[63]</sup>. There is evidence in dogs that aggressive individuals have lower cerebrospinal levels of 5-hydroxyindole acetic acid and homovanillic acid, the major metabolites of serotonin and dopamine respectively<sup>[64]</sup>.

Based on current studies, dopamine is the neurotransmitter most involved in aggression. Different breeds appear to have genes that are active at different points in the pharmacodynamics of the catecholamine. For example, compared to their non-aggressive counterparts, aggressive English Cocker Spaniels have significantly different



alleles for a dopamine receptor as well as a serotonin receptor. The gene for a dopamine receptor also appears to affect impulsive behavior in working German Shepherds, and the dopamine transporter appears to be involved in aggression, at least in the Malinois. In addition, the short form of the tyrosine hydroxylase gene appears to be involved in dopamine synthesis in German Shepherds and Siberian Huskies with particular behaviors. These studies will be discussed in detail below.

### Brain receptors for neurotransmitters

The amygdala is a structure in the brain that is associated with fear. The basolateral nuclear group of the amygdala is involved directly in the modulation of aggressive behavior in dogs. This structure has an increased volume and a higher number of neurons in aggressive dogs<sup>[65]</sup>. Serotonin 1B receptors act as auto-receptors regulating serotonin release. Indirect immunohistochemistry revealed that aggressive dogs had a higher number of serotonin 1B receptors than non-aggressive dogs. One might have expected the number to be lower in aggressive dogs, but one possible explanation is that a lower serotonergic activity is present in aggressive dogs because stimulation of presynaptic serotonin-1 autoreceptors causes a reduction of the serotonin release<sup>[65]</sup>.

Substance P is a neuropeptide that stimulates defensive aggression in cats<sup>[66]</sup> and mice<sup>[67]</sup>. It binds preferentially to neurokinin receptors. Using immunohistochemistry, Jacobs *et al.*<sup>[65]</sup> found that although the brains of aggressive dogs had more neurokinin reactivity in the amygdala than did normal dogs, the numerical densities and fractions of receptor-positive neurons did not differ significantly between the two groups. As noted above aggressive dogs have 27% more neurons in the amygdala than do normal dogs<sup>[65]</sup>.

Vermeire *et al.*<sup>[68]</sup> found differences in serotonin 2A receptors in the brains of impulsively aggressive dogs compared to normal dogs. Aggressive dogs had higher binding indexes for serotonin 2A receptors in the frontal and temporal cortex as revealed by SPECT following a 5-hydroxytryptophan (5-HT) antagonist radioligand injection. Although expensive and technically difficult, SPECT could be used to confirm a diagnosis of impulsive aggression<sup>[68]</sup>.

The brains of aggressive German Shepherds were compared with those of non-aggressive dogs of the same breed for beta adrenergic and serotonergic receptors using radioligand binding assays<sup>[69]</sup>. More binding of low affinity 5-HT (serotonergic) receptors were found in the whole brains of aggressive dogs. High affinity 5-HT was greater only in the hypothalamus and thalamus of the aggressive dogs. One might have expected 5-HT receptors to be decreased in aggressive dogs however, the increase in the number of 5-HT receptors may be due to a decrease in physiological serotonin levels at synaptic clefts or to an altered turnover of the neurotransmitter<sup>[69]</sup>.

It is not surprising that the adrenergic neurotransmitter norepinephrine might be involved in aggression.

Badino *et al.*<sup>[69]</sup> found that beta adrenergic binding was decreased in the frontal cortex, hippocampus, and thalamus of aggressive dogs. The decrease in beta adrenergic concentrations observed in these brain regions of aggressive dogs may be explained by a prolonged stimulation exerted by the high catecholamine levels resulting in beta adrenergic receptor down-regulation<sup>[69]</sup>.

In summary, there are differences in the brain, blood and cerebrospinal fluid between aggressive and non-aggressive dogs. Serotonin and its metabolites have been investigated most thoroughly. In general, blood serotonin levels are low and its metabolites are lower in aggressive dogs. The studies of receptors in the brain present a more complicated picture with serotonin receptors higher in aggressive dogs.

## HUMAN-DIRECTED IMPULSIVE AGGRESSION

### Heritability

Pérez-Guisado *et al.*<sup>[70]</sup> investigated the heritability (the percent variability due to genetics) of aggression in English Cocker Spaniels. They found that in addition to sex and coat color, nurture also influenced whether or not a dog was aggressive. The variance due to the sire heritability of aggression was only 0.2 (20%) whereas that due to the dam was 0.46 (46%) indicating a maternal-environmental effect<sup>[70]</sup>.

Although commonly perceived as gentle, non-aggressive dogs, Golden Retrievers can be aggressive, especially in European populations. Linamo *et al.*<sup>[71]</sup> used the Restricted Maximum Likelihood method to determine heritability of aggression based on the dog owner's impression of the animal's human and dog-directed aggression or the responses on C-BARQ<sup>[71,72]</sup>. They found heritability of 0.77 for human-directed aggression and 0.81 for dog-directed aggression. There is little correlation between the two types of aggression indicating separate genetic causes of the traits. There were high heritability estimates on several C-BARQ items such as strange dog approaching leashed dog (0.85), family member grooming dog (0.83), family member removing food (0.95), and stranger trying to touch dog (0.99)<sup>[71]</sup>. The next step in researching the etiology of aggression is to determine which mutations in the neurotransmitter, its receptor, or its transporters might be involved in aggression or other behavior abnormality.

### Genes

van den Berg *et al.*<sup>[73]</sup> did an extensive study of the genetic differences in four candidate genes affecting serotonin in aggressive and non-aggressive Golden Retrievers. They used mutation screens, linkage analysis, an association study, and a quantitative genetic analysis. There were no systematic differences in the coding DNA sequence of the candidate genes in aggressive and non-aggressive Golden Retrievers. An affected-only parametric linkage analysis revealed no strong major locus effect on human-



directed aggression related to the candidate genes. An analysis of 41 SNPs in the 1 Mb regions flanking the genes in 49 unrelated human-directed aggressive and in 49 unrelated non-aggressive dogs did not show association of SNP alleles, genotypes, or haplotypes with aggression at the candidate loci. They completed their analyses with a study of the effect of variation in the candidate genes on a collection of aggression-related phenotypic measures. The effects of the candidate gene haplotypes were estimated using the Restricted Maximum Likelihood method, with the haplotypes included as fixed effects in a linear animal model. They found no effect of the candidate gene haplotypes on a range of aggression-related phenotypes<sup>[73]</sup>.

Hejjas *et al.*<sup>[74]</sup> genotyped police and pet German Shepherd Dogs and diagnosed hyperactivity and impulsivity based on questionnaires. They compared the dopamine D4 receptors subtypes 2/2 with 2a/3a and 3a/3a (combined because 3a/3a is rare) with the behaviors. There was no difference in the activity-impulsivity scores between dogs with 2/2 genotype *vs* the 2/3a and 3a/3a combined genotype group either in the total sample or in the pet dog group. In contrast, police dogs with 2/2 genotype showed significantly lower activity-impulsivity scores compared with police dogs with 2/3a or 3a/3a genotype<sup>[74]</sup>.

Kubinyi *et al.*<sup>[75]</sup> found that German Shepherds with the short form of the tyrosine hydroxylase (*TH*, the enzyme involved in dopamine formation) gene were more active and impulsive. Wan *et al.*<sup>[76]</sup> also found that Siberian Huskies with the short form of the *TH* gene were more impulsive. They also reported that Siberian Huskies possessing at least one short dopamine D4 allele displayed greater activity-impulsivity in the behavioral tests than did those with two long alleles; dogs with the short allele tended to receive higher ratings on the activity-impulsivity scale of the questionnaire<sup>[76]</sup>.

Våge *et al.*<sup>[77]</sup> have used English Cocker Spaniels, a breed in which aggressive behavior has been noted for the past forty years. By using one breed, breed differences in the genotype can be eliminated so that any differences found should reflect differences in temperament. In a study comparing non-aggressive English Cocker Spaniels with English Cocker Spaniels that had bitten and broken skin, there were significant associations between aggression and four SNPs in the region of the dopamine D1 receptor (*DRD1*), two SNPs in the serotonin 1D receptor (*HTR1D*), and five SNPs in a glutamate receptor (*SLC6A1*)<sup>[77]</sup>.

The same laboratory later identified 62 SNPs occurring in or in the close vicinity of 16 neurotransmitter-related genes. Allelic associations with aggression were identified for *DRD1*, *HTR1D*, *HTR2C* (5-HT receptors D1 and 2C) and *SLC6A1* (solute carrier family 6 neurotransmitter transporter gamma amino acid member). Risk or protective haplotypes for aggressive behavior based on 2-5 SNPs were identified. The frequency of aggressive dogs varied significantly between the haplotypes within loci, and the odds ratios of aggression

in dogs with risk haplotypes compared with protective haplotypes varied from 4.4 (*HTR2C*) to 9.0 (*SLC6A1*). No haplotypes in complete association with the recorded phenotypes were identified, supporting a complex inheritance of aggression. Gene *SLC6A1* on chromosome 20 should be investigated in association with aggression in other breeds, and use of benzodiazepines which bind with gamma amino acid receptors should be investigated further as treatments for aggression<sup>[78]</sup>.

Most dogs are homozygous for the dopamine transporter-variable number tandem repeat two-tandem-repeat allele (2/2). The one-tandem-repeat allele is over-represented in American Malinois, both as heterozygotes and homozygotes (1/2 or 1/1). All American Malinois with reported seizures were 1/1 genotype. Those with at least one “1” allele (1/1 or 1/2 genotype), were more likely display hypervigilance and exhibit episodic aggression as well as more fearful postures<sup>[78]</sup>.

### Methylation

Although the genome acts as a blueprint for the production of observable morphological, physiological, and behavioral characteristics (*i.e.*, the phenotype), the expression of these traits may vary in different social or ecological contexts and in generations. Environmentally-induced phenotypic variation resulting from differential gene expression may be regulated by processes that do not include the DNA sequence itself (*i.e.*, “epigenetic mechanisms”). DNA methylation is one such epigenetic mechanism that allows organisms to respond to environmental change *via* changes in gene expression that alter the phenotype. DNA methylation during development and early life can have long-term consequences for gene expression, physiology, and behavior in many vertebrates. This is a completely uninvestigated subject in canine behavior.

## CONCLUSION

In the last ten years, the field of canine behavioral genetics has experienced rapid and exciting scientific advances, especially after completion of the sequencing of the dog genome. Although the history of dog domestication in terms of time and location is still debated, the divergence of dogs from wolves based on friendliness towards humans clearly has been outlined and experimentally repeated in the tame fox experiment. Genetic research also has focused on the great diversity of dog breeds, the genetic differences between breeds, and normal and abnormal behavioral traits. While much progress has been made in elucidating the genetics underlying aggression in dogs, future scientific studies will continue to examine this most serious problem threatening the human-canine bond and expand our knowledge about the genetic basis of canine behavior.

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