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Man's Best Friend Becomes Biology's Best in Show: Genome Analyses in the Domestic Dog*

Heidi G. Parker[1], **Abigail L. Shearin**[1,2], and **Elaine A. Ostrander**[1]

Elaine A. Ostrander: eostrand@mail.nih.gov

[1]Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892

[2]University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania 19104

Abstract

In the last five years, canine genetics has gone from map construction to complex disease deconstruction. The availability of a draft canine genome sequence, dense marker chips, and an understanding of the genome architecture has changed the types of studies canine geneticists can undertake. There is now a clear recognition that the dog system offers the opportunity to understand the genetics of both simple and complex traits, including those associated with morphology, disease susceptibility, and behavior.

In this review, we summarize recent findings regarding canine domestication and review new information on the organization of the canine genome. We discuss studies aimed at finding genes controlling morphological phenotypes and provide examples of the way such paradigms may be applied to studies of behavior. We also discuss the many ways in which the dog has illuminated our understanding of human disease and conclude with a discussion on where the field is likely headed in the next five years.

Keywords

GWAS; linkage disequilibrium; genomics; canine; domestication; complex traits

> "All knowledge, the totality of all questions and all answers is contained in the dog."
>
> – Franz Kafka

INTRODUCTION

History of the Domestic Dog

Dogs and humans have been traveling the globe together since prehistoric times. It has been so long in fact that neither of us remembers exactly how or when we met. With the genomic tools in place to trace common haplotypes, build pedigrees, and use molecular methods to disentangle ancestries, we are driven to understand the beginnings of our relationship with the domestic dog, in hope that it will provide illumination as to our own ancestors' travels, evolution, and genetics.

DISCLOSURE STATEMENT

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Fossil evidence places dogs in close proximity with humans as early as 31,000 years before present (YBP) in an Aurignacian period cave located in what is now Belgium (47). Additional evidence was found in France placing dogs with humans 26,000 YBP, and dog fossils have been found in Russia dated approximately 13,000–17,000 YBP (46, 131). In all of these sites, the dog fossils are as large or larger than modern wolves, making the classification as domestic dog difficult and controversial. Whether these fossils represent the earliest domestic dogs or extinct varieties of wolves, multiple pieces of evidence suggest that dog-like animals and humans have shared space for over 30,000 years and that the dog-wolf ancestor showed much greater variability than do modern wolves.

Molecular calculations regarding the time since domestication vary greatly and tend to agree only that the critical events occurred prior to 15,000 YBP. Mitochondrial DNA (mtDNA) analyses have suggested 100,000 YBP, 40,000 YBP, and 16,300 YBP (114, 134, 156). Comparisons of nuclear genomic data from wolves, dogs, and coyotes suggest a possible domestication time of 18,000–27,000 YBP (9000 generations) (88). All of these estimates require assumptions to be made regarding the number of independent events, the size of the critical population, mutation rate since domestication, and degree of backcrossing between domestic and wild progenitors, which is hypothesized to be extensive given the diversity that exists in modern dogs. The fact that the genome of modern domestic dogs contains both dog-specific alleles and haplotypes derived from wolves further supports the backcrossing hypothesis (5, 157).

Although phylogenetic studies based on several independent molecular datasets agree that the gray wolf is the direct ancestor of the dog, the details remain elusive (2, 39, 88, 156). mtDNA analyses of a large number of Asian dogs compared with dogs from around the world indicate a high level of diversity in the East Asian population, suggesting that that area was a major site for critical domestication events (114, 134). However, studies of African village dogs reveal a level of heterogeneity similar to that found in Asia, suggesting that a detailed analysis of other indigenous populations may yield novel theories (17). An early Asian domestication fits well with the introduction of old world dogs into the new world via migration across the Bering strait 12,000–14,000 YBP (86), but it does not explain the near simultaneous appearance of domesticated dogs throughout geographically distinct areas of Europe. Such an event would require dogs to travel without human companions, bringing into question their domestic status.

We showed in a genome-wide haplotype analysis of 912 domestic dogs and 225 wolves collected from all over the world that most modern domestic breeds share genetic signatures, to the greatest extent, with wolves from the Middle East (158). However, breeds like the Akita, which are believed to have originated in Asia, share a disproportionate number of haplotypes with Chinese wolves, whereas European breeds such as the Staffordshire bull terrier share more haplotypes with European wolves. Overall, these data suggest that wolves from many geographical regions contributed to the development of the modern dog, either through backcrossing and/or multiple domestication events (156, 158) (Figure 1).

Small dogs, morphologically distinct from wolves, were first found in human proximity in burial sites in the Levant area of Israel, south of the Mediterranean, dating approximately 12,000 YBP (147). At this point in time, and in this region, little confusion exists as to what is dog and what is wolf, as the size and shape of both had changed considerably from earlier fossil remains. It is therefore interesting that the haplotype at the insulin-like growth factor 1 (*IGF1*) locus that Sutter et al. associated with small size in the domestic dog (143) is found exclusively in wolves from this region of the world (51). This unexpected finding suggests that the genetic variants at *IGF1* that contribute to small size, a feature often associated with domestication, likely developed in the Middle East. In an independent line of study,

evidence suggests that the retrogene responsible for chondrodysplasia, which defines breeds such as the dachshund, basset hound, and corgi, also arose in this region (118), implying that the Middle East was critical for breed development through selection of specialized morphologic traits.

Sequence analysis of mtDNA from ancient dogs and wolves reveals haplotypes that are not present in modern populations of either, indicating a loss of diversity in both species since the division (47, 154). These changes affect our ability to develop precise timelines and trees describing the domestication events and subsequent evolution of the dog. As a result, there remain unanswered questions regarding dog domestication. For instance, we do not know what effect domestication has on the genome of an organism. It has been suggested that there is a relaxation of selection after domestication, leading to the build-up of mutations that would otherwise be cleared through purifying selection (13).

This idea fits well with our perception of dogs and wolves. Domestication would likely change the dog's living environment and food source, which could alter their energy consumption, change behavioral patterns, and allow less wilderness-ready individuals to thrive and reproduce under human protection. In addition, constant contact with roving human groups would have had a drastic effect on canine diversity through selective breeding, migration, hybridization, invasion, and decimation or assimilation of local populations (95). One can easily imagine the result would be the development of large numbers of nonlethal mutations for breeders to select from. Though these changes may greatly affect our ability to develop a precise timeline of domestication, they do create the opportunity to identify critical genetic changes that occurred to create our treasured modern companions. The domestic dog, then, holds a unique position as the darling of geneticists studying variation, who seek to discover genes and sequence-level changes associated with morphology, susceptibility to simple and complex diseases, and behavior.

CANINE POPULATION STRUCTURE

In 70 AD, Columella identified three specific types of dog: the guard dog, the shepherd, and the hunting dog that "does not help the farmer but actually lures him away from his work." This was the first written description of dog proto-breeds, with sizes, shapes, and behaviors related to human needs. But it wasn't until 200–300 YBP that the majority of dog breeds were established leading to the approximately 400 varieties that compose today's modern population.

In the early 1900s, registering bodies such as the American Kennel Club (AKC) in the United States and the Kennel Club in the United Kingdom were created (4). Rules were developed to further separate breeds by requiring the parents of each new puppy to be a registered member of the same breed in order for the puppy to be eligible for registration. In addition, well-documented standards were established, requiring specific size, shape, color, and sometimes behavior from each qualifying dog (4). As a result, each dog breed represents an isolated breeding population, with a constellation of traits maintained under strong selection that define each breed (110, 112) (Figure 2). Nowhere else but among domestic dogs can members of the same species coexist that differ in size by more than 40-fold as do the largest breeds, such as the Great Dane, and the Chihuahua, which is among the smallest of the breeds (112, 161).

Studies using microsatellite markers have shown that each breed has a unique genetic signature based on allele frequency and distribution that allows for singular identification of nearly every breed (116, 117). Cluster analysis of microsatellite data from as few as five dogs per breed allows breeds to be clustered into five major groups characterized by shared ancestry, usually following geographic, morphologic, and/or occupational boundaries (116,

117). These findings have recently been further refined using haplotype data constructed from approximately 48,000 single-nucleotide polymorphisms (SNPs). Phylogenetic analysis of haplotype sharing using 80 breeds with approximately 12 unrelated individuals per breed divides the population of modern dogs into approximately eight groups, each of which share common signatures of variation across the genome (158).

Not only is there a tremendous amount of population structure found between breeds, but stratification within breeds exists as well. Such variation often results from breed popularity and popular sire effects, as well as pressure on the part of the breeding community to propagate certain traits (degree of spotting, fur length, tail or ear position, etc.). By way of example, Mosher et al. identified strong signals of nonrandom mating and stratification based on racing performance levels in a study of whippets segregating a mutation in the myostatin gene (*MSTN*) that increases muscle mass (103). In a homozygous state, the mutation creates an excessively muscled dog that has an increased tendency to cramp when the muscles are stressed. In a heterozygous state, however, the musculature is only mildly exaggerated, and there is a measureable and reproducible positive effect on running speed (103). The investigators found that there was a preference for crossing dogs with the mutation within racing lines versus those that lacked the mutation in order to produce faster puppies with a more muscled appearance. This lack of random mating created substructure in the breed apparent in regions separate from the original mutation (103).

Within-breed stratification has also been described in the context of geography (126). Quignon et al. studied several breeds from Europe and the United States, assessing allele frequency and distribution of 722 SNPs from four unlinked loci (126). They found that stratification depends on population size and breeding strategies, with some breeds having experienced sufficient outbreeding to exist as a single breed across continents, whereas others are as divergent as distinct breeds. Also, Bjornerfeldt reported breed stratification based on color preferences in the poodle (12). The use of the poodle breed to map a disease gene would therefore require careful correction for the overrepresentation of one color within selected datasets.

CANINE GENOME ARCHITECTURE

Genes and the Sequence

When the sequence of the boxer was released in 2005 (88), it was estimated that the dog genome contained 19,300 genes, which is less than estimates from other sequenced species (currently 22,320 in human, 23,062 in mouse, and 24,147 in zebrafish; Ensembl database (http://www.ensembl.org). To determine if the lower numbers reflected a true loss of genes in the dog or, rather, an artifact of sequence assembly, Derrien et al. (35) examined 400 missing genes through multiple species alignment among dog, human, rat, and mouse. They compared orthologous genes and built multiple pairwise synteny maps that allowed them to infer short orthologous intervals that targeted a putatively missing canine gene (35). These data were also compared with the deep radiation hybrid (RH) map of the dog, upon which sequences from nearly 10,000 genes were localized (63).

Of the 400 missing genes that were the primary focus of the study, 70% were found, but had been miscalled. Another 12% could not be found due to poor sequence or assembly problems in the syntenic regions. Sixty-nine genes were recorded as lost because they were either totally undetected or had become pseudo-genes (35). Based on accumulation of mutations within the latter category, these genes are estimated to have lost function as many as 170 mya, well before the division of the order Carnivora from other mammalian groups (39). Although it is possible that the loss of these genes provides a selective advantage in

dogs and other carnivores, it is more likely that these findings reflect a loss of redundancy in the genome.

Linkage Disequilibrium

The creation of the breeds has altered the genome structure of the dog, affecting linkage disequilibrium (LD), haplotype structure, heterozygosity, and possibly even the rates of mutation (13, 33, 95). LD, the nonrandom association of alleles at two or more loci, can be greatly altered by population bottlenecks, small numbers of founders, and admixture. Two studies, the first by Sutter et al. in 2004 (144), followed by Lindblad-Toh et al. in 2005 (88), measured LD from five and ten loci in multiple breeds. Both studies demonstrated that, on average, LD in dogs is as much as 100-fold greater than that observed across the human population. As a result, genome-wide association studies (GWAS) in the dog require only tens of thousands of markers compared with the million or so needed for comparable human studies (88, 144). Lindblad-Toh and colleagues also calculated LD across breeds and found levels to be similar to human (~10 kb), suggesting that short-range LD in the dog arises from the ancient bottleneck created by domestication and is shared among the breeds, whereas long-range LD is the product of recent breed creation (88). An extension of the LD studies into a larger number of dog breeds shows a range of within-breed values from 20 kb in the Labrador retriever to 4.5 Mb in the Pekingese (50), although measures were variable across the genome (88, 144). Not surprisingly, LD in dog breeds displays significant correlation with demographic history, although there are outliers: breeds that display higher or lower levels of LD than expected based on population history (Figure 3). This demonstrates the complex factors contributing to breed formation, including hybridization, migration, and evolving selective pressures.

Interestingly, Gray et al. detected a similar range of values in a diverse collection of wolf populations. As with dogs, the degree of LD correlated closely with both population size and severity of bottleneck (50). Although dog populations are considered artificial because of man’s interference, they can still provide accurate models of extreme events in natural populations and inform genome-based conservation efforts.

Haplotype Structure

The haplotype structure of the dog was elucidated in conjunction with the prior LD analyses (88, 144). In ten regions dispersed over the genome and spanning approximately 100 kb each, Sutter et al. reported that less than three haplotypes made up the majority of chromosomes in each breed. In addition, only 4.5 haplotypes were found in 80% of chromosomes in all five breeds tested, and on average, each breed pair shared more than 50% of their haplotypes (144). The degree of sharing reflected the common history of some breeds. For instance, the Labrador and golden retriever breeds shared the highest number of haplotypes, and the Japanese Akita and the Swedish Bernese mountain dog, which have no known common heritage, shared the fewest. As is evident in the following sections, these findings have strong implications for fine mapping loci of interest using combinations of breeds that share a trait from a common origin.

Homozygosity and Selection

In the analysis of the 7.8x boxer sequence, Lindblad-Toh and colleagues noted extensive regions of homozygosity interspersed with highly heterozygous regions along each chromosome (88) (Figure 4). The homozygous regions were on average sixfold longer than the heterozygous regions and covered 62% of the boxer genome. Closer examination suggests that extensive stretches of homozygosity exist in all dogs, with distinct patterns for each breed (88).

Based on the assumption that artificial selection has shaped the canine genome, Akey et al. examined 21,000 SNP genotypes from 10 breeds and assessed signatures of selection through examination of population differentiation statistics in one Mb windows across the genome (3). Out of 1933 windows, 155 showed significant homozygosity in at least one breed. Examination of some of those regions revealed genes that were plausible candidates for breed specific traits. For example, one such region in the Chinese shar-pei breed contained an intronic deletion associated with skin wrinkling in the HSA2 locus, which contains the gene for hyaluronic acid synthatase. Interestingly, excessive wrinkling of the skin, which typifies the shar-pei breed, correlates with increased serum hyluronic acid levels (166).

Canine SNP Resources

In 2005, the 7.8x genome sequence of a domestic dog, a boxer, was made available (88), following release of the 2x sequence of the standard poodle (82). The combination of these two resources, along with one Mb of sequence from nine additional breeds and a group of wild canids, generated more than 2.5 million SNPs that could potentially be used for trait mapping (88). To date, four whole genome SNP chips have been made available [Affymetrix, Santa Clara, CA (http://www.affymetrix.com); Illumina, San Diego, CA (http://www.illumina.com)], with increasing numbers and quality of SNPs on each new release.

CAUSES OF GENOMIC VARIATION

Retrotransposons

In 1992, Minnick and colleagues identified the first canid-specific repeat element, a widely dispersed short interspersed nuclear element (SINE) (101). Termed SINEC_Cf, these repeats represent about 7% of the dog genome sequence (82) and are highly active. Indeed, more than 6000 SINEs were found to be heterozygous in the canine reference sequence alone (88). Comparing the boxer and poodle reference sequences, Kirkness et al. reported 11,000 bimorphic SINEC_Cf elements and predicted that another 10,000 are likely to exist. These numbers are in stark contrast to the less than 1000 active SINEs estimated in the human genome (96). In addition to the SINE population, long interspersed nuclear elements (LINEs) are more active in the dog than in humans, and evidence suggests that SINEs are transposing additional flanking chromosomal sequences in conjunction with the retrotransposons (81).

Polymorphic SINEs have already been implicated in both morphology and disease in the dog (Table 1). For instance, Clark et al. showed that insertion of a SINE element into the SILV gene causes merle coat patterning in the Shetland sheepdog (31).

Copy Number Variation

In 2004, two large-scale human genome analyses revealed that copy number varations (CNVs), duplications or deletions of entire stretches of genomic DNA, are common throughout the normal human genome and are a major source of individual variation (reviewed in 167). To date, more than 38,000 CNVs have been identified in the human genome, and some of the most exciting studies in the past year are those that tested for an association between CNVs and phenotype.

Multiple approaches have been used to assess CNV in the dog genome. The first two efforts, that of Chen and colleagues (29) and Nicholas et al. (107), both assessed multiple different breeds compared with the boxer. Chen and colleagues used a commercially developed whole genome aCGH array, whereas Nicholas et al. developed a custom aCGH array based on

segmental duplications predicted from the canine sequence. Chen identified 155 CNVs in nine dogs with an average size of approximately 300 kb and found multiple indications for breed or breed-group specificity. Nicholas's targeted approach enabled the development of a denser array that identified 3,583 CNVs in 678 unique regions from 17 domestic dogs and a wolf. The average size of the CNVs was 33 kb. Approximately 38%, however, could not be assigned to a chromosome using the current CanFam2 assembly. Both studies agree that larger numbers of dogs and markers would be needed to develop an accurate picture of CNV patterning in the domestic dog.

A study by J.D. Degenhardt, E. Karlins, A. Auton, T.C. Spady, P. Quignon, et al. (unpublished data) utilized much greater numbers of dogs. This study assessed CNVs in 781 dogs from 75 breeds at approximately 125,000 markers based on intensity data from the Affymetrix v.2 canine SNP chip. By increasing the number of dogs, the authors were able to identify 9,789 CNVs in 1,220 regions. Approximately 8% of the CNVs had been identified previously in one of the two earlier studies, whereas more than 90% were unique and 76% overlapped genes (J.D. Degenhardt, E. Karlins, A. Auton, T.C. Spady, P. Quignon, et al., unpublished data). Analysis of the SNPs surrounding the CNVs showed a marked decrease in LD compared with LD observed between SNPs. In addition, many of the CNVs were not shared by closely related breeds, suggesting that they are frequently recurring events, more likely to share identity by state rather than by descent.

Slippage

Arguably, the most interesting questions in canine research today are centered on an effort to understand the source of the phenotypic variation that characterizes breeds. The active SINE family may play a role in increasing the frequency of nonlethal mutations available for breeders to select on, as may a relaxation of selective pressures (33, 81). Fondon and colleagues, however, propose that an abundance of repetitive elements in developmental genes may provide the answer (43). They identified polymorphic repeats in the coding sequence of 36 developmental genes likely to be important in morphologic evolution and compared them with orthologous repeats in human genes. Indicated by expansion or contraction of the repeat, they found a significant increase in selection of divergence in 29 of the 36 dog genes examined in at least a subset of breeds. Variation in the number of unit repeats in the coding regions of the *Alx-4* (aristaless-like 4) and *Runx-2* (runt-related transcription factor 2) genes were specifically associated with significant differences in limb and skull morphology (43).

In a subsequent study, Laidlaw et al. sequenced 55 coding repeat regions in 42 species representing 10 major carnivore clades and found that dogs possess a genome-wide increase in the basal germ-line slippage mutation rate compared with other Carnivoran families and primates (85). In addition, when comparing orthologous microsatellite sequences in dog and human, they found that the reported increase in purity in dog repeats is a genome-wide phenomenon and is not specific to a few genes (85). This implies that the increase in slippage rates may have contributed to the malleability of the canine genome and, as such, could account for the high level of phenotypic variation in breeds.

MORPHOLOGY AND BREEDS

Identifying genes that are responsible for the morphologic features that define breeds is a major goal for many in the canine genomics community. Thus far, interest has focused on genes that control body size, leg length, and variations in coat. In each case, not only have the genetic underpinnings been unraveled, but contributions have been made to understanding mammalian developmental biology as well.

Body Size

The first efforts to find loci associated with body size were undertaken by Chase et al. in the Portuguese water dog (PWD) breed (27). These researchers sampled DNA and a set of five radiographs from over 500 dogs. From the radiographs they derived a set of 92 skeletal metrics that they used to establish phenotypes for a linkage study. Key to their success was a principal component analysis (PCA) used to identify groups of traits that were coregulated. They mapped several sets of traits, with the strongest result, PC1, describing overall body size. PC1 is now known through analyses of several datasets to be controlled by four to six loci (16, 71), the strongest of which was initially defined as a 4 Mb region on canine chromosome 15 (CFA15).

Sutter et al. followed up this preliminary observation by analyzing CFA15 in large and small PWDs and a multi-breed dataset of large and small breeds (143). Although the four million bases included several genes, the presence of a selective sweep in 14 small breeds highlighted *IGF1* as the causative gene. Interestingly, analysis of multiple small breeds, like the toy poodle and Pomeranian, revealed that most individuals were homozygous for an identical haplotype, suggesting that the critical mutation(s) likely occurred early in the domestication process (143). When large breeds, such as the St. Bernard and Newfoundland, were examined, two other haplotypes dominated, offering other routes for the *IGF1* gene to increase skeletal size.

The results of the *IGF1* study were exciting for several reasons. First, it demonstrated that the canine system was indeed sufficiently powerful to decipher the genetics of traits that have proven intractable in humans. Second, it suggested that dogs can bridge human studies, which are notoriously underpowered, and mouse studies, which often lack refined phenotypes. Finally, it demonstrated the power of the multi-breed approach (88, 110, 112) (Figure 5), which takes advantage of the fact that many mutations are likely to have occurred early in domestication, and are then propagated through the independent meiotic events leading to breed formation. This offers an efficient way to fine-map regions identified in GWAS studies. Other studies of both morphology and disease had made the latter point with regard to single gene traits (49, 73, 117), but this powerful demonstration of quantitative trait locus (QTL) fine mapping of a morphologic trait opened the door for scientists to study the genetics of literally any breed-defining trait.

In order to create a dataset that would allow us to tackle this problem on a large scale, approximately 900 dogs representing 80 breeds were genotyped using the Affymetrix v.2 canine SNP chip, generating a dataset of nearly 50,000 informative SNPs per dog (16). The number of lineages was maximized by genotyping a dozen unrelated dogs from each breed. The resulting data, termed CanMap, was analyzed to identify dozens of loci for morphologic traits, including body size, leg length and width, tail and ear position, skull shape, etc.

The Canine Coat

The genetics of coat color has been studied extensively in the dog (11, 31, 73, 76, 124, 136) and, more recently, the wolf (5). Although melanin production through the melanocortin 1 receptor (*MC1R*) controlled by agouti signal peptide (*ASIP*) has been well described in many species (69, 72, 142, 150), a recent canine study has identified a new gene in the color pathway. Black coat color in most dogs is not controlled by mutations in *ASIP* or *MC1R* (77, 78). Rather, dominant black is caused by a variant in β defensin 103 (*CBD103*), a protein previously associated with immune function (21). This finding further supports the role of melanocyte signaling in immunity and adds a new dimension to the study of coat color.

More recently, studies of canine coat length and texture, beginning with simple Mendelian traits segregating in single breeds, have been undertaken. Hillbertz et al. identified a 133 Kb

duplication on chromosome 18 that associated perfectly with the presence of the ridge of hair growing in the opposite direction along the spine of the Rhodesian ridgeback (62). In addition, Drögmüller and colleagues found a frameshift variant in the *FOXI3* gene that segregated with the hairless phenotype, also called ectodermal dysplasia (CED), in the Chinese crested breed (37). Both of these mutations have important health implications as dogs with the ridge are predisposed to a congenital abnormality called dermoid sinus, which is a neural tube defect, whereas the hairless mutation is homozygous lethal. These provide interesting examples of deleterious traits that have piggybacked with genes under selection for desirable traits. As canine researchers map additional traits under selection, the identification of other deleterious loci is sure to follow.

The most comprehensive study of canine coat structure has been undertaken by Cadieu et al., who did complimentary GWAS analyses in single breeds where specific coat types segregated and in the larger CanMap dataset, and then looked for overlapping peaks of association (20). They identified genes and variants controlling curl, length, and furnishings, which is a pattern of hair growth on the face and legs of some dogs typified by a mustache. Two of the three genes identified, fibroblast growth factor 5 (*FGF5*) and keratin 71 (*KRT71*), had been previously associated with hair growth in dog and mouse, respectively (65, 130).

However, the identification of r-spondin 2 (*RSPO2*), which is strongly associated with the furnishings growth pattern, was a surprise. A 167 bp insert in the 3′ untranslated region (UTR) of *RSPO2* was found in all dogs with furnishings in a dataset of over 1000 dogs of varying phenotypes. Although not previously associated with hair growth, *RSPO2* synergizes with Wnt to activate β-catenin (75), and Wnt signaling is required for establishment of hair follicles (6, 32). In addition, the Wnt/β-catenin pathway is involved in the development of hair-follicle tumors, or pilomatricomas (26). In dogs, these tumors occur most frequently in breeds with furnishings (100).

Examination of dogs with a variety of coat types demonstrated that variant alleles at these three genes create seven different coats and can explain 95% of the fur phenotypes observed in the nearly 1000 dogs studied (20) (Figure 6). Thus far, only a few breeds, such as the exceptionally long coated Afghan hound and the curly-coated retriever, are not explained by the three-gene model, suggesting that a small number of additional genes contributing to subtleties of fur growth remain to be found.

One of the advantages of conducting morphologic studies in dogs is that there is strong phenotypic uniformity within breeds. As a result, mapping can sometimes be done using breed average data presented through the registering kennel club as a phenotype (111). Jones et al. demonstrated this in an analysis of 148 breeds (71), identifying the same loci for body size and other morphologic features as had been published previously, but they also reported putative loci for newly considered traits such as longevity and behavior. This suggests that the dog system holds promise for understanding some of the most basic questions that occupy today’s scientists (112).

CANINE BEHAVIOR

Three different aspects of behavior in dogs have attracted modern geneticists: behaviors associated with personality, such as loyalty and protectiveness; behavioral disorders, including rage and obsessive-compulsive disease (OCD); and breed-defining behaviors, including herding, drafting, and pointing (45, 109, 119). Little is known about the genetics of personality traits in dogs. The work of Hare et al. (52) establishes that dogs are able to relate to humans in ways unique from what even primates can achieve, but the underlying

genetics has yet to be tackled in a major way. Thus far, the most is known about anomalous behaviors, as they often mimic human disorders.

Behavioral Disorders

Dogs and humans share a number of behavioral disorders, including anxiety, rage, compulsive behavior, and attention deficit disorder. Many show evidence of heritable components through pronounced breed disposition (14, 108, 113). To date, candidate gene analyses based on human studies have been the primary route to finding mutations associated with canine behavior (57, 58, 145, 149). Recently, however, the first GWAS for a behavioral disorder in dogs was completed, identifying a region on CFA7 associated with a flank-sucking compulsive disorder in Doberman pinschers (36). No mutation has been identified to date, but the most promising candidate in the region is a neuronal adhesion protein *CDH2*.

Canine compulsive disorders mimic human obsessive-compulsive disorders at a pharmacologic and phenotypic level. For instance, they respond to treatment with clomipramine hydrochloride, a serotonin-reuptake inhibitor. One of the most interesting phenotypes described in the literature is that of tail chasing, observed primarily in the bull terrier (102). It will be interesting to see if GWAS identify the same locus in the bull terrier as the Doberman, or if other genes in the relevant pathways are found.

In addition to compulsive diseases, some effort has gone into the study of hyperactivity and aggressive behavior in dogs. For instance, an association between variants in the dopamine D4 receptor gene and impulsivity was reported in the German shepherd dog (59). Aggression has been studied in several breeds based on biochemical observations. Reisner et al. (128) reported decreased concentrations of a major metabolite of seratonin in cerebrospinal fluid of dominant aggressive dogs, and Badino et al. (8) found modifications of serotonergic receptor concentrations in the brains of aggressive dogs. In response, Van den Berg et al. (152) evaluated four serotonergic genes with respect to aggressive behavior in golden retrievers. Using mutation screens, linkage analysis, association studies, and quantitative genetic analysis, no obvious associations were found. However, in a study of Shiba Inu, Takeuchi et al. reported that polymorphisms in the *SLC1A2* gene were significantly associated with aggression towards strangers (145).

One interesting phenotype that has been well described is owner-directed aggression (127), which occurs in nearly half of all springer spaniels and at a lower frequency in related breeds like the English cocker spaniel (125). Manifesting as unprovoked biting or other threatening behaviors, owner-directed aggression is the major behavioral problem many experts report they encounter. Although training and environmental factors clearly play a role, the overrepresentation of some breeds with the phenotype suggests a clear rationale for a GWAS.

Breed-Associated Behaviors

Setting aside the issue of anomalous behaviors, studies show that there are both behavior and personality traits associated with specific breeds (18, 53, 54, 132, 146). Simply put, border collies do not herd sheep because they are raised on sheep farms; rather, they are raised on sheep farms because they herd. In addition pointers point, retrievers retrieve, and mastiffs guard, all because those traits are part of their breed expectations, meaning strong and continuous selection in the underlying breeding program (Figure 2). Although one recent study has suggested genomic regions where loci may exist that are associated with some of these behaviors (71), no genes have been identified in large part because of the difficulties associated with breaking these complex traits into quantifiable phenotypes.

A promising new approach derives from a recent study of performance-related traits in racing Alaskan sled dogs (68). Racing dogs are divided into distance and sprint categories. The latter perform short (<30 mile) runs, whereas the former race for hundreds of miles over multiple consecutive days, as in the renowned Idi-tarod race. Huson et al. studied the genomes of sprint and distance racing dogs and identified purebred signatures within these highly mixed populations associated with specific traits, such as endurance, work ethic, and speed (68). Follow-up GWAS studies are now in progress to identify the underlying genes. What is likely to emerge for each trait is a complex picture of multiple interacting genes. An understanding of how the network of genes functions will establish a new paradigm for studying behavioral genetics.

CANINE DISEASE MAPPING INFORMS HUMAN DISEASE

The study of complex human disorders has been stymied by the small size of human families for linkage studies, the limited size of case groups for GWAS, and the inability to accurately phenotype many complex diseases. Attempts to stratify human conditions based on their clinical presentation, response to treatment, or long-term outcomes, thus simplifying the underlying locus heterogeneity, have been only partially successful.

Genetic studies of canine disease have been of proven value in three major ways (138). First, because human and canine disorders are similar from a medical perspective, the identification of canine disease genes often offers unique opportunities to test new therapies. Second, canine genetic studies often identify genes or gene families that were not previously associated with disease. Finally, identification of disease genes in the dog has foretold new ways in which mammalian genomes can be perturbed to produce phenotypes of interest.

Many canine diseases are caused by mutations in the same genes as the corresponding diseases in humans. Canine X-linked hemophilia A is caused by a gene inversion in factor VIII, the same gene that causes the human disease (94). X-linked severe combined immune deficiency, observed in both basset hounds and humans, is caused by a 4 bp deletion in the *IL2-R gamma* gene in dogs, which produces a truncated protein, as does the human mutation (61). Several breeds of dog share a predisposition for adult-onset insulin-dependent diabetes, including the Samoyed, Tibetan terrier, and Cairn terrier, and alleles at the canine DRB and DQA loci are associated with the disease (24). Indeed, the canine DLA locus appears to be associated with several diseases, including early-onset systemic lupus erythematosus (SLE), as seen in the Nova Scotia duck tolling retriever, which is associated with variant alleles in DLA class II genes (163). By way of a final example, mutations in the *SLC3A1* gene cause type 1 cystinuria in humans and a similarly severe form of the disease in the Newfoundland (60).

Canine Disease Studies Offer Relevant Animal Models for Human Disease

Cancer and autoimmune disease in the dog often recapitulate human disorders so closely that they become models for the development of new therapies. Cancer generally occurs spontaneously in the dog, with a clinical presentation, histology, disease progression, and response to treatment similar to human, e.g., transitional cell carcinoma of the bladder, non-Hodgkin's lymphoma, chronic myelogenous leukemia, osteosarcoma, and melanoma (19, 48, 104, 115). In addition, several breeds exhibit a strong genetic predisposition for a particular cancer, suggesting a genetic component. For instance, Scottish and West Highland white terriers have a high incidence of transitional cell carcinoma of the bladder (48); the Scottish deerhound, Irish wolfhound, and Rottweiler frequently get osteosarcoma (138); and mammary cancer is often diagnosed in English springer spaniels (40).

In some cases, an understanding of the underlying genetics has begun, and it is likely that the dog condition will be useful for informing us about comparable human diseases. For instance, canine mammary cancer in English springer spaniels from Sweden shows an association with the *BRCA1* and *BRCA2* loci (129). Two loci for Addison's disease have been mapped in the Portuguese water dogs; one is in the DLA region, the other is near the immunosuppression locus *CTLA-4* (28).

In the case of Duchenne X-linked muscular dystrophy, the dog model, golden retriever muscular dystrophy (137), is so similar to the human disease that multiple labs are actively studying it. The disease is caused by loss of the dystrophin protein, a critical component of the dystrophin-glycoprotein complex (64). In dogs, the underlying mutation is in a consensus splice acceptor site, leading to exon 7 skipping, disruption of the open reading frame, and premature termination of translation (66, 137). In contrast to an existing mouse model, the affected dogs are analogous to humans displaying progressive muscle wasting, with degeneration and fibrosis. As a result, they are frequently used to test novel therapeutic interventions, often via bone marrow transplant, that may prove useful for human patients (reviewed in 160).

Canine Disease Genetics Highlights New Genes and Pathways

Retinitis pigmentosa, the leading cause of blindness in both purebred dogs and humans, is a constellation of diseases that in the dog is referred to as progressive retinal atrophy (PRA). Although multiple causative genes have been described in man and dog (reviewed in 122), many forms of the disease in both species remain unmapped. Of interest to researchers in both fields is the identification of causative genes in the canine that were not previously recognized as contributors to the human disease. Progressive rod-cone degeneration (prcd), a late onset form of the disease, affects multiple breeds, including poodles, cocker spaniels, and Labrador retrievers. A linked marker was found in 1997 (1), and the region was narrowed to 106 Kb through LD mapping in multiple breeds after several painstaking years (49) (Figure 5). The underlying canine mutation, which occurs in a novel 54–amino acid protein, was described shortly thereafter (165) and appears to be important for both canine and human disease.

Although useful for identifying specific disease genes, canine mapping studies are even more useful for revealing whole pathways relevant to a human condition. The best example is that of narcolepsy in the Doberman pinscher, which is caused by a fully penetrant autosomal recessive SINE insertion in the hypocretin/orexin receptor 2 gene (*HCRTR2*) (25, 70, 87). Human narcolepsy is rarely attributed to a mutation in the *HCRTR2* gene but is commonly caused by a decreased number of hypocretin cells. The hypocretin/orexin signaling pathway is implicated as the decreased cell number likely results from a disrupted hypocretin/orexin pathway (25). This was the first gene identified that conferred a risk for a sleep disorder, highlighting a pathway that had not previously been implicated in the studies of human sleep.

The same argument can be made regarding copper toxicosis, which was mapped in the Bedlington terrier breed (164), and is caused by a mutation in the *MURR1* gene, now called *COMMD1* (151). Although virtually nothing was known about the gene when it was linked to copper toxicosis in 2002, it is now recognized as a regulator of copper metabolism (34, 44).

Canine Mapping Studies Reveal New Ways In Which the Genome Can Be Altered

Finally, canine studies suggest new ways in which mutations can change phenotype. For instance, studies of autosomal recessive progressive myoclonic epilepsy (PME) in the

miniature wire-haired dachshund (MWHD) reveal a disease very similar to human Lafora disease, which is caused by mutations in the EPM2 genes: *EPM2a* and *EPM2b* on HSA 6q22–24 (140). Lohi et al. (92) mapped a locus for PME in the MWHD to CFA35 in a region syntenic to HSA 6p21–25. Sequencing of canine *EPM2b* revealed a dodecamer repeat at the 5′ end that normally exists in 2–3 copies. MWHD exhibiting PME, however, possess 19–26 copies of this repeat. Subsequent studies revealed a 14-copy repeat in an affected basset hound. The mutation apparently affects expression, as *EPM2b* mRNA levels in the skeletal muscle of affected dogs are expressed at levels 900 times lower than what is observed in normal canine skeletal muscle (92). Although expansions of trinucleotide repeats have been associated with human disease, this is the first example of a dodecamer repeat causing a disorder.

Even studies of morphology reveal new disease mechanisms. For example, Parker et al. recently showed that expression of a retrogene encoding a complete copy of the coding sequence of fibroblast growth factor 4 (*FGF4*) is strongly associated with chondrodysplasia, the short-legged phenotype that is a requirement for approximately 20 breeds including the dachshund, corgi, and basset hound (118). Interestingly, the retrogene has none of the original regulatory machinery, but genes surrounding it are expressed in fetal chondrocytes, as is the retrogene. Expressed retrogenes have been found in insects, but this is the first time, to our knowledge, that one has been identified that controls a major phenotype in a mammal. Not only is this gene now a candidate for cases of human asymmetrical dwarfism in which the phenotype is not explained by known genes, but the novel disease mechanism must now be considered in cases where sequencing of candidate genes does not reveal underlying mutations (118).

CONCLUSION

The American writer, Corey Ford, tells us that, “Properly trained, a man can be dog’s best friend.” If that is the case, then in the last several years, canine researchers have distinguished themselves. We have sequenced the genome of the dog (88) and found the mutations causing dozens of diseases, and, as a result, genetic tests are widely available for many diseases.

Consequently, we have come to recognize the enormous value of the canine system for understanding the human species. We have demonstrated that many of the genes causing dog disorders cause similar diseases in humans, begun to unravel the genetics of a multitude of complex traits, and discovered new ways in which the genome can be perturbed to cause changes in phenotype. At the most fundamental level, we have undertaken comparative genomics, discovering how subtle changes in the genome cause a wealth of changes in the species, offering a smorgasbord of variations for breeders to select from as they propagate existing breeds and develop still newer varieties. We now understand why big breeds are big and little ones are small, and we will, shortly, understand much more about the genetics of the canine form.

Our primary goals for the next five years are threefold. First, we will continue to disentangle the genetics of complex traits and use the canine system to understand the genetics of common diseases like heart disorders and diabetes. Second, there remains in the 400 or more dog breeds an extraordinary amount of untapped variation, simply because the phenotypic measures have not been developed to quantify it. As they become available, we can expect to see the canine genetic system applied to the study of everything from tail length to metabolic rate. Finally, there remain to be studied truly complex quantitative traits such as behavior and response to drug therapies. As we develop an ever more sophisticated

understanding of genetic variation in the canine genome, the genes controlling those traits will be found as well.

In the end, however, what will be the most difficult to understand is our own relationship with the dog. Unwavering loyalty, compassion, and blind adoration are not traits we can map with our genomic tools, and for now we must be content with that. In the words of American author Margery Facklam, “We give dogs time we can spare, space we can spare, and love we can spare. In return, dogs give us their all. It’s the best deal man has ever made.”

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Glossary

Haplotype	combination of alleles at multiple loci on the same chromosome that segregate together
YBP	years before present
Mitochondrial DNA (mtDNA)	circular molecule of DNA contained within the mitochondria of the cell rather than the nucleus and inherited through the maternal lines
Allele	one specific variation at a site where there are two or more possibilities (polymorphic site)
Locus	a specified position in the genome
Single-nucleotide polymorphism (SNP)	one nucleotide position in a DNA sequence that has more than one possible base
Linkage disequilibrium (LD)	Nonrandom association of alleles from adjacent loci on a chromosome; caused by a variety of reasons and indicates reduction in recombination between the loci
Genome-wide association study (GWAS)	a scan of the entire genome using a large number of markers in multiple unrelated individuals designed to identify regions segregating with measurable traits
Genotype	the combination of two alleles carried by an individual at a particular locus
Short interspersed nuclear element (SINE)	a very common type of small (100–500 bp) retrotransposon
Long interspersed nuclear element (LINE)	a large retrotransposon that carries a promoter and codes for the proteins required to replicate and reinsert itself into the genome
Copy number variation (CNV)	section of genomic DNA that has undergone duplication or deletion
Phenotype	an observable and/or measurable trait

Principal component analysis (PCA)	mathematical method for grouping correlated variables to create uncorrelated sets, reducing the complexity of a dataset while minimizing information loss
Polymorphism	variation in DNA sequence from a reference sequence; location in the DNA sequence where more than one allele exists in a population

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SUMMARY POINTS

1. An extraordinary level of variation is captured within the dog genome. Although we don't yet understand how such variation occurred, studies of dog breeds offer the opportunity to understand how combinations of variants create complex phenotypes.
2. Studies of canine skeletal variation promise to expand the vocabulary of genes associated with growth, and in turn, diseases of growth regulation.
3. The availability of the dog genome sequence has made possible studies of genome architecture that have expanded our understanding of linkage disequilibrium, repeat elements, and gene organization.
4. Canine behavioral genetics is in its infancy, but studies are initiating that will permit us to understand how genetics contributes to both normal and anomalous behaviors.
5. Canine and human diseases are remarkably similar in terms of phenotypic presentation and causative genes. As such, the dog serves as a system for understanding human diseases that have otherwise proven difficult to study.

FUTURE ISSUES

1. We need to develop or improve methodologies in genomics and behavioral phenotyping to make use of the canine genome project to identify genes controlling both anomalous and naturally occurring behaviors.
2. We must use our new found knowledge to find genes important in complex traits, particularly diseases that are common in the human population, such as cancer, diabetes, and heart disease.
3. We must work to connect the dots created by individual morphological studies in order to understand the ways in which genetic variants combine to produce the amazing variety observed in domestic dog breeds.
4. In addition to the genomic advances, we need to focus on assembling the tools needed to undertake studies aimed at understanding the role of epigenetic events in phenotypic variation.

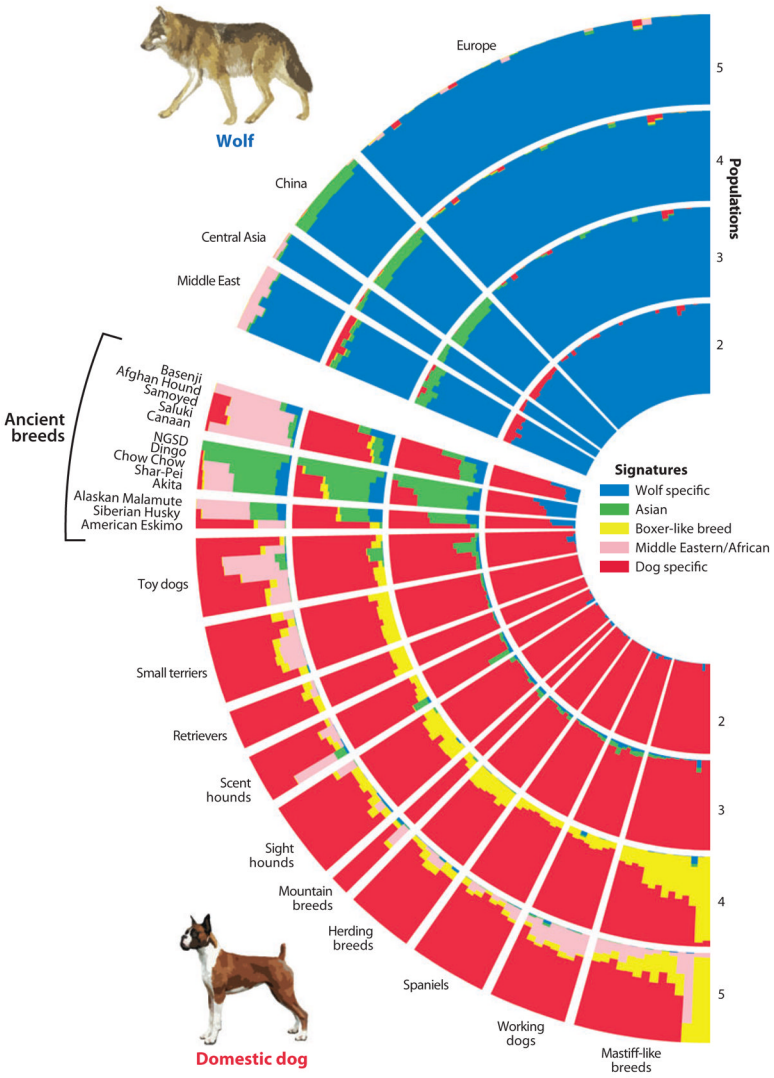

Figure 1.

An unsupervised cluster analysis of dogs and wolves. Using clustering algorithms with more than 43,000 single-nucleotide polymorphisms (SNPs), 85 dogs, representing 85 different breeds, along with 43 wolves from Europe and Asia, were assigned to 2–5 populations (*inner circle* to *outer circle*, respectively) based solely on genomic content. Each column represents a single individual divided into colors representing genomic populations. Blue indicates a wolf-specific signature, and red indicates a dog-specific signature. Note that the majority of crossover lies between ancient dog breeds and Chinese or Middle Eastern wolves. Figure originally published in *Nature* (158).

Figure 2.
Variation in dog breeds encompasses a large range of sizes, shapes, colors, and behaviors. Breeds (and behaviors) are listed from the upper left, clockwise: Border collie (herding: controlling the movement of livestock is one of the oldest described behaviors in dogs), Pug dog (companionship: one of the most prized traits a dog provides), Whippet (coursing: chasing small or large game, a natural forerunner to racing), Alaskan sled dogs (mushing: pulling a sled with driver as a member of a team for either sport or transportation), German shorthaired pointer (pointing: identifying the location of game), Foxhounds (tracking: working as a pack to follow scent of moving game and signal the hunter), Portuguese water dog (water retrieval: used on fishing boats to bring in nets and line, round up fish, and deliver messages between boats), Bernese mountain dog (drafting: pulling heavy loads individually or in pairs, usually to transport goods). Herding picture provided courtesy of paurian@flickr.

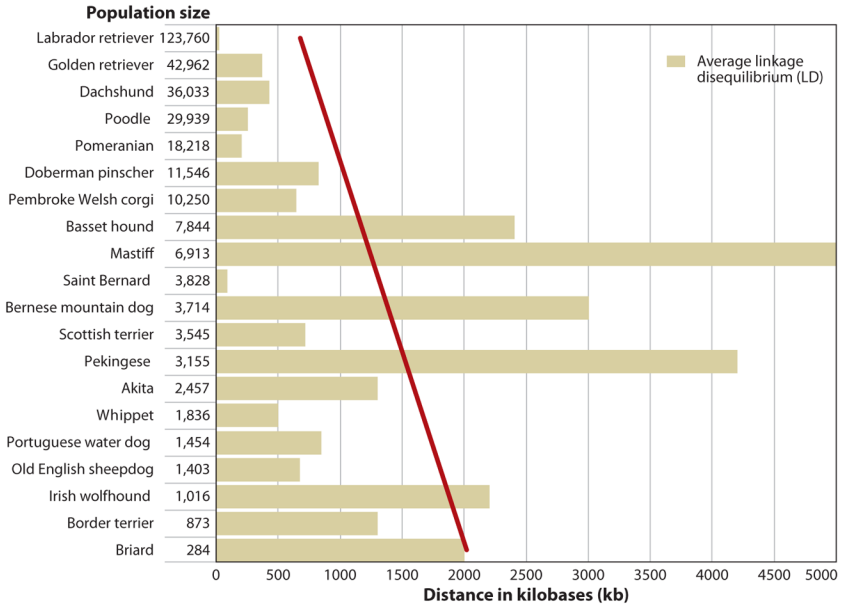

Figure 3.

Average linkage disequilibrium (LD) in 20 dog breeds sorted by breed population size. The breeds are listed at the left of the graph, followed by the number of dogs registered in the breed in 2009. LD was calculated at five unlinked loci by Sutter et al. and Gray et al. (50, 144). Linear trend line indicates overall tendency for LD to increase with a decrease in population size. Breeds that display considerable deviations from the trend likely have complex population histories involving multiple changes in population size, admixture, and/or changing selective pressures.

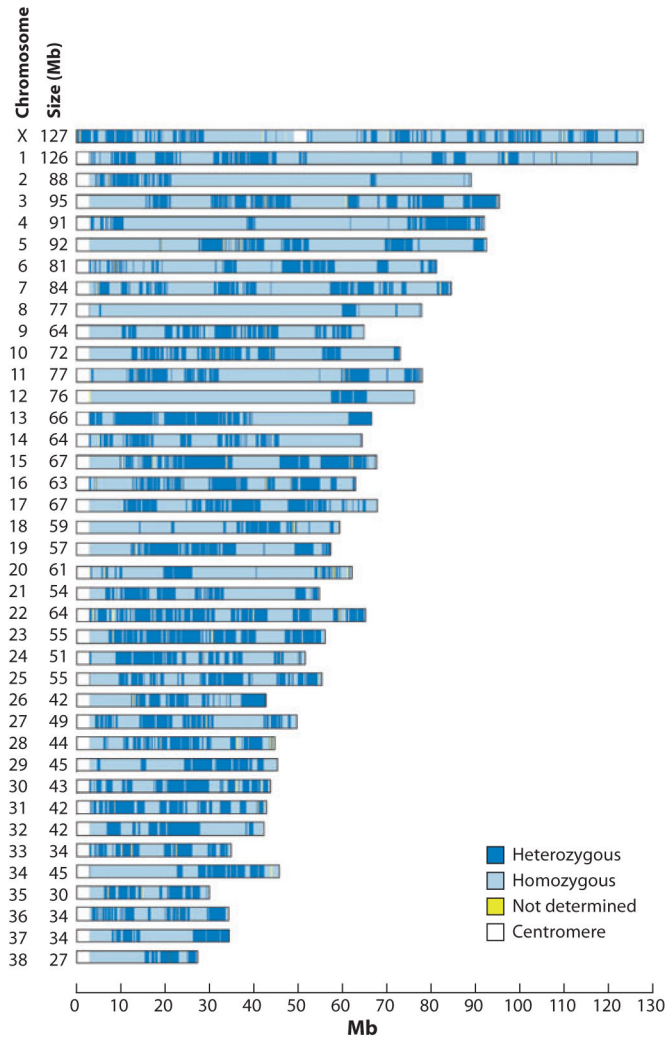

Figure 4.

Regions of homozygosity and heterozygosity as found through direct sequencing of the boxer genome. All 38 autosomes and the X chromosome were sequenced from a female boxer to produce a 7.8x draft sequence. Heterozygous or homozygous state of each contig in the genome build was assessed though examination of approximately 770,000 single-nucleotide polymorphisms (SNPs) with heterozygous = 1 SNP in 1000 bps versus homozygous = 1 SNP in 20,000 bps. Contigs with the same state were merged to form blocks. Average size of the light-blue homozygous blocks is 6.9 Mb. Average size of the dark-blue heterozygous blocks is 1.1 Mb. White blocks indicate the centromeres. All canine chromosomes except the X are telocentric. Figure originally published in *Nature* (88).

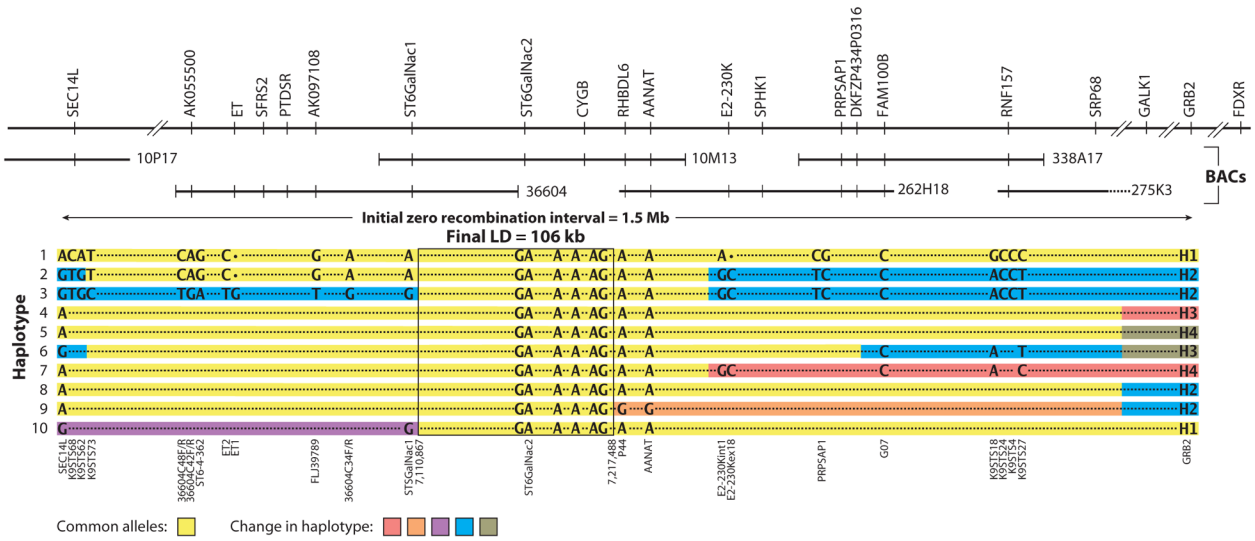

Figure 5.

Haplotype shared among ten breeds of dog reduced the progressive rod-cone degeneration (prcd) locus from 1.5 Mb to 106 Kb. An identical haplotype spanning 106 Kb was found in miniature and toy poodles, English cocker spaniels, American cocker spaniels, Labrador retrievers, Portuguese water dogs, Chesapeake Bay retrievers, Nova Scotia duck tolling retrievers, Australian cattle dogs, and American Eskimo dogs with allelic forms of prcd. The causative mutation was identified in a novel gene, now called *PRCD*, located between *CYGB* and *ST6GALNAC2* (165). The top row shows a schematic of the chromosome region with genes named above. Below the chromosome are the bacterial artificial chromosomes (BACs) used to tile sequence across the interval. The haplotypes are shown with common alleles colored yellow, and a change in color indicates a recombination event or change in haplotype. Solid dots indicate deleted nucleotides. Names of tagging markers used for genotyping are given at the bottom of the haplotype chart. Figure originally published in *Genomics* (49) and reprinted with permission from Elsevier.

	Phenotype	*FGF5*	*RSPO2*	*KRT71*
a	Short	–	–	–
b	Wire	–	+	–
c	Wire and curly	–	+	+
d	Long	+	–	–
e	Long with furnishings	+	+	–
f	Curly	+	–	+
g	Curly with furnishings	+	+	+

Figure 6.
Seven different coat phenotypes are created through the allelic variation at three genes. Protein altering mutations in *FGF5* and *KRT71* along with changes in expression levels of *RSPO2* combine to create the seven coat types displayed. The combinations of alleles are displayed to the right of the coat type. The coat types represented by each breed are as follows; (*a*) short, (*b*) wire, (*c*) curly-wire, (*d*) long, (*e*) long with furnishings, (*f*) long and curly, (*g*) long and curly with furnishings. Figure originally published in *Science* (20).

Table 1

Examples of molecular mechanisms associated with phenotypes in the dog

Class	Trait	Discovery method	Reference
Single base change: coding			
Morphology	Curly hair	GWAS	(20)
Morphology	Long hair	GWAS	(20)
Morphology	Brown coat[a]	Linkage	(136)
Morphology	Red/yellow coat	Candidate gene	(42, 106)
Morphology	Black mask	Candidate gene	(135)
Morphology	Recessive black coat	Candidate gene	(77)
Morphology	Fawn/sable coat[b]	Candidate gene	(11)
Disease	Degenerative myelopathy	GWAS	(7)
Disease	Renal cystadenocarcinoma and nodular dermatofibrosis	Linkage	(90)
Disease	Progressive rod-cone degeneration	Linkage	(165)
Disease	Neonatal encephalopathy	Linkage	(30)
Disease	Exercise-induced collapse	Linkage	(120)
Disease	Narcolepsy (DACH)[c]	Candidate gene	(67)
Disease	Cystinuria	Candidate gene	(60)
Disease	Cone degeneration (GSHP)[c]	Candidate gene	(139)
Disease	Dominant progressive retinal atrophy	Candidate gene	(79)
Disease	Shaking puppy (generalized tremor)	Candidate gene	(105)
Disease	Rod-cone dysplasia 1	Candidate gene	(141)
Disease	Glanzmann's thrombasthenia (OTTR)[c]	Candidate gene	(15)
Disease	Dystrophic epidermolysis bullosa	Candidate gene	(9)
Disease	Von Willebrand's type 2 (GWHP)[c]	Candidate gene	(83)
Disease	Hemophilia B	Candidate gene	(41)
Disease	Leukocyte adhesion deficiency	Candidate gene	(80)
Disease	Alport syndrome	Candidate gene	(168)
Single base change: noncoding			
Morphology	Dilute pigment	Linkage	(38, 124)
Disease	X-linked ectodermal dysplasia	Linkage	(23)
Disease	Narcolepsy (LAB)[c]	Candidate gene	(87)
Disease	Muscular dystrophy (GOLD and CKCS)[c,d]	Candidate gene	(137, 159)
SINE insertion: coding			
Disease	Centronuclear myopathy	Linkage	(121, 148)
SINE insertion: noncoding			
Morphology	Merle (patches of dilute pigment)	GWAS	(31)
Disease	Narcolepsy (DOBP)[c]	Linkage	(87)

Class	Trait	Discovery method	Reference
Insertion or deletion (not SINE): coding			
Morphology	Hairless	GWAS	(37)
Morphology	Dominant black coat	Linkage	(21)
Morphology	Brown coat[a]	Candidate gene	(136)
Morphology	Myofiber hyperplasia “Bully whippet”	Candidate gene	(103)
Disease	Copper toxicosis	Linkage	(44, 164)
Disease	Neuronal ceroid-lipofuscinosis	Linkage	(74, 89)
Disease	Cone degeneration (AMAL)[c]	Linkage	(139)
Disease	Imerslund-Grasbeck disorder	Linkage	(55, 56)
Disease	Cone-rod dystrophy (MLHD and SWHD)[c,e]	Linkage	(99, 162)
Disease	Rod-cone dysplasia type 2	Linkage	(84)
Disease	X-linked severe combined immunodeficiency	Candidate gene	(61)
Disease	von Willibrand’s disease type 3 (SCOT)[c]	Candidate gene	(153)
Disease	Ivermectin sensitivity	Candidate gene	(97)
Disease	Hereditary cataracts (STAF)[c,f]	Candidate gene	(99)
Disease	Early onset cataracts (BOST)[c,f]	Candidate gene	(98)
Disease	Congenital night blindness	Candidate gene	(155)
Disease	Recessive progressive retinal atrophy	Candidate gene	(123)
Disease	Glanzmann’s thrombasthenia (GPYR)[c]	Candidate gene	(91)
Disease	Cyclic hematopoiesis	Candidate gene	(10)
Insertion or deletion (not SINE): noncoding			
Morphology	Furnishings	GWAS	(20)
Disease	Collie eye anomaly	Linkage	(93, 117)
Disease	Glanzmann’s thrombasthenia (GPYR)[c]	Candidate gene	(91)
Disease	Junctional epidermolysis bullosa	Candidate gene	(22)
Expanded repeat: coding			
Disease	Myoclonus epilepsy	Linkage	(92)
Gene or segmental duplication/ rearrangement			
Morphology	Ridgeback	GWAS	(133)
Morphology	Chondrodysplasia	GWAS	(118)
Disease	Hemophilia A	Candidate gene	(94)
Disease	Chronic myelogenous leukemia	Candidate gene[g]	(19)

SINE, short interspersed nuclear element; GWAS, genome wide association study.

[a] One deletion and two SNPs have been associated with brown coat color.

[b] Two SNPs in complete linkage disequilibrium are associated with fawn/sable coat color.

[c] Breed abbreviation key: AMAL-Alaskan malamute, BOST-Boston terrier, CKCS-Cavalier King Charles spaniel, DACH-dachshund, DOBP-Doberman pinscher, GOLD-golden retriever, GPYR-great Pyrenees, GSHP-German short-haired pointer, LAB-Labrador retriever, MLHD-

miniature long-haired dachshund, OTTR-otterhound, SCOT-Scottish terrier, STAF-Staffordshire bull terrier, SWHD-standard wire-haired dachshund.

[d] Different SNPs in the same gene are associated with muscular dystrophy in the GOLD and CKCS.

[e] Insertions in two different genes are responsible for cone-rod dystrophy; *RPGRIP1* in the MLHD and *NPHP4* in the SWHD.

[f] The same mutation was found associated with early onset cataracts in the BOST and hereditary cataracts in the STAF.

[g] Fluorescent in situ hybridization with probes from a candidate chromosomal region was used to identify the rearrangement that causes CML.