Review

Inbreeding and genetic diversity in dogs: Results from DNA analysis

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ABSTRACT

This review assesses evidence from DNA analysis to determine whether there is sufficient genetic diversity within breeds to ensure that populations are sustainable in the absence of cross breeding and to determine whether genetic diversity is declining. On average, dog breeds currently retain approximately 87% of the available domestic canine genetic diversity. Requirements that breeding stock must be 'clear' for all genetic disorders may firstly place undue genetic pressure on animals tested as being 'clear' of known genetic disorders, secondly may contribute to loss of diversity and thirdly may result in the dissemination of new recessive disorders for which no genetic tests are available.

Global exchange of genetic material may hasten the loss of alleles and this practice should be discussed in relation to the current effective population size of a breed and its expected future popularity. Genomic data do not always support the results from pedigree analysis and possible reasons for this are discussed.

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Introduction

The literature relating to canine diversity and inbreeding within and across breeds can be interpreted in the context of our modern appreciation of genomic data and recent understanding of the domestication of dogs from the grey wolf. In this light, genomic data can be used to show the current status of within-breed diversity in the dog. The process of domestication has some bearing on the expected degree of canine diversity existing across all breeds. This will be influenced in part by the number of wolf founders.

There has been considerable debate over how and when dogs were domesticated. Early studies suggest that dogs were first domesticated in South-East Asia. It has been postulated the dog was one of the first domesticated species (Sundqvist et al., 2006), but the number of domestication events could not be pinpointed with accuracy in the absence of broad sampling of dogs across Asia, the Middle East and Europe. Other studies have suggested Africa or the Middle East as centres of domestication (Wayne and Ostrander, 1999; Boyko et al., 2009). New comprehensive mitochondrial evidence suggests a single point of origin in South-East Asia, possibly by humans in agricultural communities engaging in early rice growing (Pang et al., 2009). Since domestication, dogs have travelled with humans throughout the world. It is now known that dogs were domesticated from as many as several hundred wolves (based on mitochondrial evidence) but in a single location now confirmed as being South-East Asia (Pang et al., 2009).

Observations of mitochondrial diversity and maternal genetic diversity

Using mitochondrial evidence, domestic dogs cluster into three major population clades, denoted A–C (Vila et al., 1997; Savolainen et al., 2002). The proportions of domestic dogs within these clades are similar, even in dispersed regions (Pang et al., 2009). Mitochondrial DNA exists separately from nuclear DNA and is overwhelmingly maternally inherited. Unlike the nuclear genome (which is present as two copies in normal individuals) many hundreds of mitochondrial sequences can be present in a single individual. This hardy DNA can be detectable even in grossly degraded specimens (Webb and Allard, 2010).

To date, 217 mitochondrial haplotypes have been identified in the domestic dog (Webb and Allard, 2010) and these are clustered into ten haplogroups (Pang et al., 2009). Many kinds of dogs have contributed to this sampling, ranging from village dogs to pedigree show dogs. The mitochondrial groupings allow for six sub-clades within clade A and two each within clades B and C.

To survey more than 95% of the haplotypes likely to exist within a given population, it has been recommended that more than 450 dogs need to be sampled (Webb and Allard, 2010). Only one mitochondrial diversity study (Pang et al., 2009) has sampled at a sufficient density to yield meaningful demographic data (Webb and Allard, 2010): 1543 dogs were sampled from across Asia and Europe, along with 33 dogs from Arctic America and 40 Eurasian wolves. Typically, such data are used to estimate the number of population founders, as well as the timing and nature of population bottleneck events. The results suggest that dogs were domesticated in southern China between 5400 and 16,300 years ago from at least 51 female wolf founders.

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All 10 haplogroups remain in dog populations at the putative site of domestication. As dogs have dispersed from this region in their travel with humans, fewer haplogroups are represented, with seven present in Central China and only four in Europe. This pattern of decreasing haplotype diversity from the point of origin has been observed in other species, including humans (International Hapmap Consortium, 2005).

The projected number of wolf founders of domestic dogs is 51 if the foundation event was closer to 16,000 years ago and could be as high as several hundred maternal founders if domestication was at the recent end of the projected range. This difference in estimates arises because time allows new mutations to accumulate, providing an alternative explanation for the diversity of haplotypes observed. It should be recognised that even founder females are likely to have carried a mixture of mitochondrial haplotypes.

Individual dog breeds cannot be readily distinguished by mitochondrial sequence information (Sundqvist et al., 2006; Parra et al., 2008), further supporting a common ancestral origin for most dog breeds (Vila et al., 1997, 1999; Savolainen et al., 2002). Most dog breeds have a mixture of divergent mitochondrial haplotypes and these often represent different mitochondrial clades (A–C). Breeds sharing common mitochondrial haplotypes rarely have similar phenotypes.

Information on paternal diversity using Y chromosome sequences

To complement our understanding of maternal inheritance, it is useful to examine the genomic signatures of paternal lineages among domesticated dogs and to investigate the potential effect of founder males across and within dog breeds. Bannasch et al. (2005) tested paternal diversity using Y chromosome microsatellites in 824 unrelated males from 50 breeds of domestic dogs. The study identified 67 haplotypes across all breeds. On average, 36% of the variance in haplotypes occurred within breeds, while 67% of variance occurred across breeds. Comparable Y chromosome microsatellite haplotype variances for 20 dispersed human populations are 23% between populations and 77% within populations (Kayser et al., 2001).

Twenty-six breeds of dogs had unique haplotypes and 15/50 breeds had no Y chromosome diversity (a single haplotype was represented). A significant number of haplotypes was shared across breeds, indicating a common ancestry for the breeds sharing haplotypes. The number of haplotypes observed across breeds exceeded the number observed using autosomal microsatellites by approximately two-fold (Parker et al., 2004). In contrast with the evidence from mitochondrial DNA, the deepest Y chromosome divergence was exhibited by dogs from Africa.

Sundqvist et al. (2006) studied four Y chromosome microsatellites in 20 breeds of dogs, each represented by five male individuals. Their findings support those of Bannasch et al. (2005) that breeds are typically founded by few males and that a large proportion of breeds have limited or no Y chromosome diversity. In five pointing dog breeds, four Y chromosome haplotypes were identified among five breeds: German shorthaired pointer ($n = 10$), Deutch Drahthaar ($n = 4$), Espaneul Breton ($n = 7$), English pointer ($n = 27$) and English setter ($n = 42$) (Parra et al., 2008). Four Y chromosome microsatellites were tested. One haplotype was fixed in the Deutch Drahthaar, whereas other haplotypes were shared between at least two breeds and a single haplotype was present in all breeds examined.

Information on diversity from autosomal microsatellite markers

In the first study comprehensively to examine genomic structure in purebred dog populations, Parker et al. (2004) genotyped 96 microsatellites from 414 purebred dogs representing 85 breeds; the sampled dogs (five per breed) shared no common grandparents. Between-breed variation accounted for 27% of observed autosomal microsatellite variance. This result contrasts strongly with the results from microsatellite analysis of the Y chromosome (Bannasch et al., 2005), suggesting that the majority of autosomal diversity is maternally derived. The comparable across-population autosomal diversity for humans is 5–10% (Parker et al., 2004).

On the basis of microsatellite genotype alone, 99% of dogs could be correctly assigned to breed and Asiatic breeds were the most divergent (Parker et al., 2004). This analysis showed that there has been strong isolation of pedigree dog populations. Despite this, the study found that the phylogenetic breed topology was flat for breeds derived in Europe, suggesting a common ancestry. In contrast, the African breeds (represented by the Basenji) and Asiatic breeds formed distinct branches in the phylogenetic tree.

Expected heterozygosities for each of the 85 canine breeds analysed by Parker et al. (2004) were estimated by Tajima's unbiased estimator using the average of 96 microsatellite loci (Tajima, 1989). The expected within-breed heterozygosities (based on observed allelic frequencies) ranged from 0.31 (Bedlington terrier) to 0.61 (Australian Shepherd). However, this is likely to be underestimated because of the low number of dogs ($n = 5$) sampled for each breed.

Single nucleotide polymorphism studies

Using end-sequencing of bacterial artificial chromosomes (BAC) by PCR, Parker et al. (2004) sequenced 19,867 nucleotides in each of 120 dogs, representing 60 breeds. Seventy-five polymorphic sites were identified, each with one single nucleotide polymorphism (SNP) per 264 bp; of these, 14/75 (19%) were each limited to one breed (having resulted either from genetic drift or new mutations).

As part of the canine genome project, 561 SNPs were examined in 20 individuals from each of eight breeds across 10 random genomic regions (denoted as SNP set 1) (Lindblad-Toh et al., 2005). Combining all eight breeds and using Tajima's method applied to the data from Parker et al. (2004), the heterozygosity was calculated as 0.322, while within-breed values ranged from 0.219 for the Akita ($n = 11$) to 0.299 for the Labrador retriever ($n = 20$) (K. Lindblad-Toh, personal communication). The analysis carried out as part of the canine genome project found that we can expect 70% of loci validated as polymorphic in a large across-breed analysis to be polymorphic within any other single breed (Lindblad-Toh et al., 2005).

As part of the quality-control analysis for the Affymetrix version 1.0 canine genotyping array, Karlsson et al. (2007) observed within-breed heterozygosities in the range of 0.252 (Akita) to 0.280 (Leonberger) when sampling 10 individuals per breed. Across breeds, the observed heterozygosity was 0.283, which is lower than the figure from the genome analysis, most likely because there were fewer individuals sampled within each breed.

Across-breed analyses make use of SNPs that demonstrate polymorphism and are validated through genotyping based on real data. Such polymorphic SNPs may be limited to one of the SNP-discovery breeds (Lindblad-Toh et al., 2005). This results in an ascertainment bias that reduces the apparent diversity in breeds other than the SNP-discovery breeds. Most SNPs in the public domain are derived from the Boxer, the Poodle and, to a lesser extent, other breeds, including the Labrador retriever, Alaskan Malamute, Beagle, Bedlington terrier, Italian greyhound, English shepherd dog, German shepherd dog, Portuguese water dog and Rottweiler (Lindblad-Toh et al., 2005). Therefore, it is not surprising that the Labra-