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# Unequal Contribution of Sexes in the Origin of Dog Breeds

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ABSTRACT

Dogs (*Canis familiaris*) were domesticated from the gray wolf (*Canis lupus*) at least 14,000 years ago, and there is evidence of dogs with phenotypes similar to those in modern breeds 4000 years ago. However, recent genetic analyses have suggested that modern dog breeds have a much more recent origin, probably <200 years ago. To study the origin of contemporaneous breeds we combined the analysis of paternally inherited Y chromosome markers with maternally inherited mitochondrial DNA and biparentally inherited autosomal microsatellite markers in both domestic dogs and their wild ancestor, the gray wolf. Our results show a sex bias in the origin of breeds, with fewer males than females contributing genetically, which clearly differs from the breeding patterns in wild gray wolf populations where both sexes have similar contributions. Furthermore, a comparison of mitochondrial DNA and Y chromosome diversity in dog groups recognized by the World Canine Organization, as well as in groups defined by the breeds' genetic composition, shows that paternal lineages are more differentiated among groups than maternal lineages. This demonstrates a lower exchange of males than of females between breeds belonging to different groups, which illustrates how breed founders may have been chosen.

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DOGS (*Canis familiaris*) were domesticated from gray wolves (*Canis lupus*) at least 14,000 years ago (Vilà *et al.* 1997; Sablin and Khlopachev 2002; Savolainen *et al.* 2002), well before the domestication of any other animal or plant species (Clutton-Brock 1999). Genetic analyses suggest a limited number of domestication events with subsequent transcontinental spread (Vilà *et al.* 1997; Leonard *et al.* 2002; Savolainen *et al.* 2002), and archaeological evidence confirms that domestic dogs existed across at least three continents by 10,000 years ago (Schwartz 1997; Clutton-Brock 1999; Sablin and Khlopachev 2002). This relatively fast spread of dogs across continents suggests that they may have played an important role in primitive human societies.

The roles fulfilled by modern dogs are many and varied, but likely different from those in Stone Age communities. The World Canine Organization (Fédération Cynologique Internationale, FCI) currently recognizes ~347 breeds of dogs, classified in 10 groups according to their function and, to a lesser degree, area of origin (for example, group 10 contains sighthounds; see Table 2 legend). Modern breeds differ widely in size, conformation, behavior, and physiology (Hart 1995; Coppinger and Coppinger 2001; Wayne and Vilà 2001). The phenotypical differences among dog breeds exceed those among breeds of other domestic mammals and even between species in the entire family Canidae (Wayne 1986a,b).

Ancient origins have been claimed for some dog breeds (Crowley and Adelman 1998). Archaeological evidence from ancient Egypt suggests that several types of morphologically differentiated dogs (similar to mastiffs and greyhounds) existed 4000 years ago, and Romans may have been the first people to develop dog breeds in Europe as early as the first century A.D. (Clutton-Brock 1999). Dogs on paintings from the sixteenth century can be easily recognized today as spaniels, mastiffs, hounds, pointers, etc. However, this long history contrasts with the results of genetic studies, which suggest that most of the current breeds may represent a recent radiation from a common stock and that distinct breeds may have been formed from "less codified phenotypic varieties after the introduction of the breed concept and the creation of breed clubs in Europe in the 1800s" (Parker *et al.* 2004, p. 1164). The genetic comparison of dog breeds using autosomal markers shows that breeds constitute well-defined entities, differentiated from each other (Zajc and Sampson 1999; Koskinen 2003; DeNise *et al.* 2004; Parker *et al.* 2004). On the other hand, mitochondrial DNA (mtDNA) comparisons fail to show clear differentiation between breeds (Okumura *et al.* 1996; Vilà *et al.* 1997, 1999a; Savolainen *et al.* 2002). These observations, together with the difficulties in establishing a well-resolved phylogeny

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of breeds (Irion *et al.* 2003; Parker *et al.* 2004), have been taken as an indication for lack of long-term isolation between breeds and a recent origin and differentiation for most modern breeds.

To gain a better understanding of how dog breeds were formed we present here a combined analysis of genetic markers with different patterns of inheritance. Specifically, the study of paternally inherited Y chromosome markers offers a view of the foundation of dog breeds complementary to that obtained from studies using only maternally inherited mtDNA or biparentally inherited markers. We also analyze the maternally and paternally inherited marker systems in gray wolves, the ancestor of the domestic dog. Results from the wild populations of gray wolves allow us to put the dog results in perspective despite inherent differences between the markers. Combining these three marker systems allows us to assess the contribution of each sex to the origin of some breeds and provides a better understanding of the process leading to breed formation.

MATERIALS AND METHODS

One hundred male dogs representing 20 breeds (5 dogs per breed, see Table 1) were genotyped for 18 biparentally inherited autosomal microsatellites, 4 paternally inherited Y chromosome microsatellites, and the maternally inherited mtDNA control region to compare the patterns of variability within breeds for each kind of marker. A larger sample of 214 additional male dogs from 89 breeds was also typed for the Y chromosome markers to study the degree of differentiation between the groups of dogs breeds recognized by the FCI. We also typed Y chromosome microsatellites and sequenced mtDNA in male gray wolves from six different populations across North America and Eurasia (Alaska, $n = 12$; Inuvik, Canada, $n = 13$; Russia, $n = 12$; Finland, $n = 31$; Baltic States, $n = 24$; and Spain, $n = 20$) to compare the patterns of variability with those observed for the dog breeds.

Genomic DNA was extracted from blood or tissue using a proteinase K digestion followed by a modified phenol-chloroform extraction (Sambrook *et al.* 1989). Partial mitochondrial control region sequences were obtained for the samples as described in Vilà *et al.* (1997). Although sequences of ~400 bp were obtained, only the 261 bp homologous to the sequences in Vilà *et al.* (1997) were used in the analyses, and haplotypes were denoted as previously. Phylogenetic relationships between dog mtDNA haplotypes were assessed by constructing neighbor-joining phylogenetic trees using the Tamura–Nei model of sequence evolution and assuming a gamma shape parameter $a = 0.5$ in the program PAUP* 4.0b10 (Swofford 1998). From their position in the tree, sequences were identified as belonging to one of the four clades of dog sequences (I–IV) in Vilà *et al.* (1997), which have been suggested to represent different domestication events for dogs.

Four Y chromosome microsatellites were typed (Sundqvist *et al.* 2001). Since the Y chromosome does not recombine over most of its length, the combination of the alleles found at the four Y chromosome microsatellites represents haplotypes that are inherited as single units. The divergence between the different haplotypes was assessed assuming that each single repeat change in each microsatellite represents one mutational event (Ellegren 2000). A matrix of haplotype divergences (measured as number of mutational steps separating the haplotypes) was used to construct a network of haplotypes, using the program TCS 1.8 (Clement *et al.* 2000).

The 18 biparentally inherited autosomal microsatellites typed are distributed across the canine genome: c2001, c2006, c2010, c2017, c2054, c2079, c2088, and c2096 (Francisco *et al.* 1996); vWF (Shibuya *et al.* 1994); u109, u173, u225, and u253 (Ostrander *et al.* 1993); and PEZ01, PEZ03, PEZ05, PEZ08, and PEZ12 [Perkin-Elmer (Norwalk, CT), Zoogen; see the FHCRC Dog Genome Project at http://www.fhcrc.org/science/dog_genome/]. All data are available from the authors upon request. Genotypes were used to assess the degree of genome-wide similarity across all individuals. This was done by building a neighbor-joining tree based on a matrix of pairwise distances $d_{ij} = 1 - P_{ij}$, where P_{ij} is the proportion of shared alleles between individuals i and j. The genetic structure of dog breeds was also assessed by assignment tests, where the probability of finding each genotype is estimated for each one of the breeds and the animal is then assigned to the breed for which the probability is highest (Cornuet *et al.* 1999). Additionally, we used a Bayesian approach, as implemented by the software STRUCTURE (Pritchard *et al.* 2000; Falush *et al.* 2003), to assess if the dog genotypes can be clustered by breed without providing any additional information about breed affiliation as in Parker *et al.* (2004). Each individual genotype was assigned to the group with the highest probability.

We assessed the proportion of the mtDNA and Y chromosome variance distributed among the FCI groups of breeds and within them, using an analysis of molecular variance (AMOVA) approach as implemented in the program Arlequin 2.001 (Excoffier *et al.* 1992). For the mtDNA we used a published data set including haplotype information from 430 purebred dogs of breeds recognized by the FCI (Savolainen *et al.* 2002). As above, we considered the degree of divergence between the different haplotypes, correcting for multiple hits using the Tamura–Nei model of sequence evolution with a gamma shape parameter $a = 0.5$. For the Y chromosome we considered the haplotype information for the 314 purebred male dogs from this study (5 males from each of 20 breeds plus the 214 additional dogs from 89 breeds). The divergence between haplotypes was measured in two different ways that estimated the number of mutations that separate the haplotypes while correcting for the different number of alleles observed at each locus. First, we used a distance based on the infinite-allele model (Estoup and Cornuet 1999), $d_{1_{ij}} = \sum_{k=1,m}(X_{ijk}/n_k)$, where $d_{1_{ij}}$ is the distance between haplotypes i and j, m is the number of microsatellite loci studied on the Y chromosome (four in our case), $X_{ijk} = 1$ if the alleles observed at locus k in haplotypes i and j are different and 0 if they are identical, and n_k is the number of alleles observed at each one of the loci. Second, we used a distance based on the stepwise mutation model (Estoup and Cornuet 1999), $d_{2_{ij}} = \sum_{k=1,m}(Y_{ijk}/n_k)$, where Y_{ijk} is the number of mutational steps (assuming that each mutation involves the increase or decrease of the microsatellite in only one repeat unit; Ellegren 2000) separating the alleles observed at locus k in haplotypes i and j. These two distances were intended only to characterize the relative difference between the Y chromosome haplotypes under different assumptions and cannot be compared with each other and with other genetic distances. Finally, AMOVA tests were also performed, comparing actual haplotype frequencies as opposed to distances.

RESULTS

Diversity within dog breeds: The structure of a tree of the genotypes obtained from dogs from 20 breeds (Table 1) using 18 autosomal microsatellites demonstrates

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TABLE 1

MtDNA and Y chromosome haplotypes observed in dog breeds (five males per breed) and number of haplotypes observed in wolf populations and across all dogs

Dog breed /wolf population	mtDNA	Y chromosome
Dogs		
Airedale terrier	*D4*, D6, *D27*	h12, h31
Beagle	D6	h71
Bernese mountain dog	*D1*, *D9*	h39
Border terrier	*D3*, *D26*	h17
Boxer	*D3*, *D4*, *D14*	h40
Cairn terrier	*D1*, D6, *D14*	h72
Cavalier King Charles spaniel	*D4*, *D17*	h33
Collie, rough/smooth	*D1*, D6, **D28**	h39
Dalmatian	*D4*, *D16*, *D29*	h39, h71
Flatcoated retriever	*D4*, D10	h31
German pointer	*D4*, D6, *D26*	h12
German shepherd	*D4*, D10, *D26*	h12
Golden retriever	*D4*, D6	h31
Greyhound	*D3*, *D4*, *D9*	h31, h39
Irish soft-coated wheaten terrier	*D3*, D6, *D14*, *D26*	h18. h33
Newfoundland	*D4*	h31
Poodle, miniature/standard	*D3*, D6, **D7**, *D26*	h12, h39
Rottweiler	*D3*	h33, h70
Shetland sheepdog	*D2*, D6, *D26*	h39, h72, h88
West Highland white terrier	*D1*, D6, *D14*	h12, h39
Total:	15	11
Wolves		
Alaska (n = 12)	6	6
Russia (n = 12)	4	6
Inuvik (n = 13)	4	7
Finland (n = 31)	3	7
Spain (n = 20)	2	5
Baltic States (n = 24)	2	9

Dog mtDNA haplotypes in italics belong to clade I, those in boldface type to clade III, and those underlined to clade IV (mtDNA clades are as in Vilà *et al.* 1997).

the overall similarity existing among the members of each breed (Figure 1). For 14 of the 20 breeds, all 5 dogs analyzed clustered in a single group, and for all other breeds at least 3 of the dogs formed a single clade. However, this representation does not completely portray the differentiation between breeds: assignment tests allowed the correct identification of the breed to which 96 of the 100 dogs belong. Additionally, a Bayesian approach showed that this small number of microsatellites was enough to genetically characterize the breeds and when the dog genotypes were sorted in 20 groups (number of populations $K = 20$) each group corresponded to 1 breed and 94 dogs were correctly assigned to their breed. The 6 individuals that were misassigned included one Newfoundland, two German pointers, one golden retriever, and two poodles. Interestingly, the two poodles that were not correctly assigned were miniature, while the rest were standard, which indicates that the two groups of animals are genetically differentiated and the two mismatches may not correspond to classifications to a wrong breed. In all cases the misassigned individuals were assigned to the wrong breed with a low probability.

In contrast, breeds were not well separated for mtDNA haplotypes. Multiple mtDNA types were observed for most breeds (Table 1). These haplotypes were often very different from each other and in many cases they originated from different clades representing different lineages at the time of domestication (Vilà *et al.* 1997, 1999a; Savolainen *et al.* 2002). For example, in five collies we observed three different sequences belonging to three different clades (Table 1). At the same time, breeds sharing a certain mtDNA haplotype were not necessarily phenotypically similar to each other. For instance, haplotype D3 was found in breeds as different as the boxer, the West Highland white terrier, the cairn terrier, the poodle, and the greyhound. Together with other studies (Vilà *et al.* 1997, 1999a; Savolainen *et al.* 2002) this shows an overall lack of structure in the mtDNA of dog breeds.

On the other hand, between two and six mtDNA haplotypes were observed within the wolf populations. The mtDNA haplotypes observed in wolves were different

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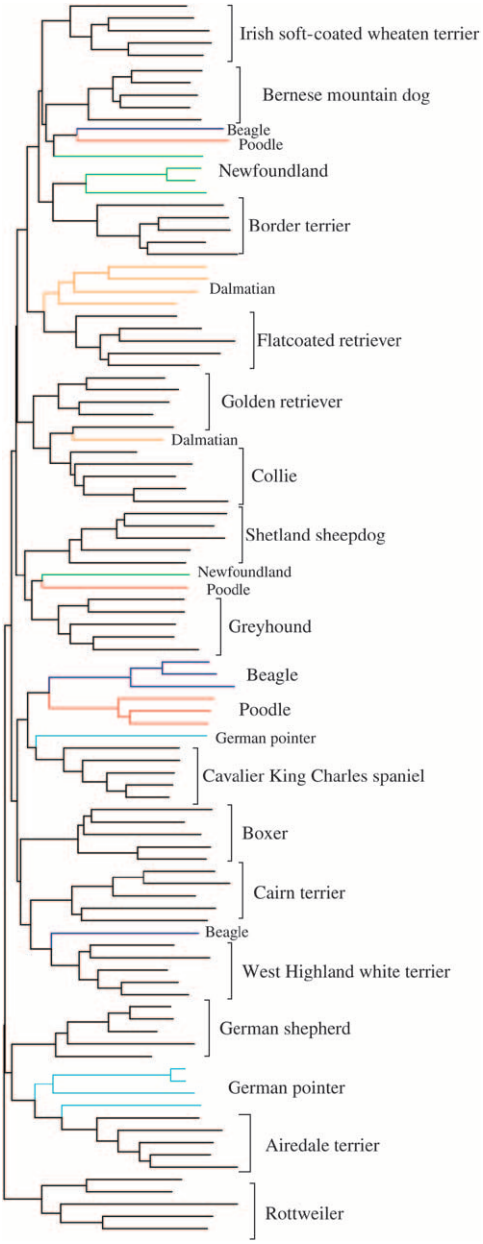

Figure 1.—Neighbor-joining tree of individual genotypes based on 18 autosomal microsatellites. Individuals from the same breed are enclosed in a bracket if they cluster in the same area of the tree or are marked with the same color if they are located in different parts of the tree.

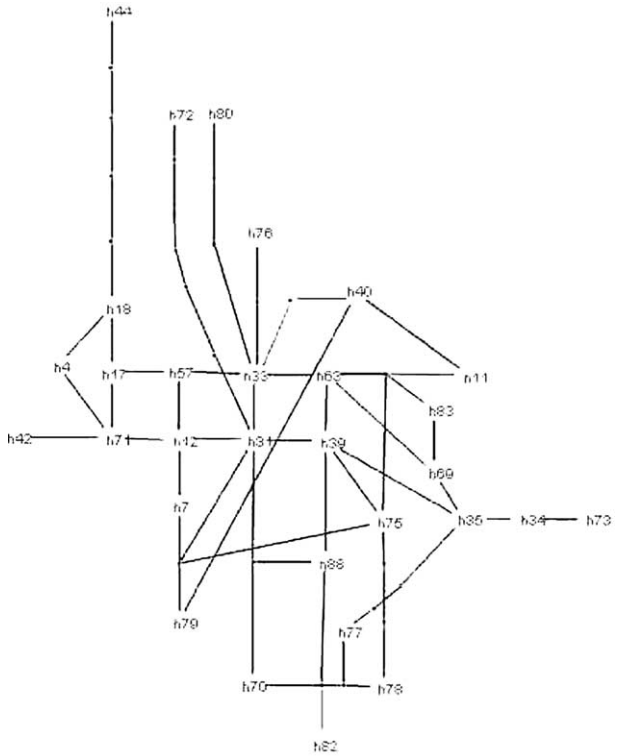

Figure 2.—Haplotype network of Y chromosome haplotypes. Each branch represents one mutational step. Hypothetical haplotypes are indicated with dots.

from those in dogs. The poor phylogeographic patterns in the distribution of mtDNA control region variation in wolves (Vilà *et al.* 1999b) and the fact that we have sampled just a few populations [none of them in Asia, which harbors >50% of the wolves in the world (Boitani 2003)] imply that associations between the observed wolf and dog haplotypes cannot be used to infer the origin of the dog diversity.

A lack of structure between breeds was also exhibited by the paternally inherited Y chromosome markers. While for 12 of the 20 breeds for which five males had been studied only one Y chromosome haplotype was observed (Table 1), some haplotypes were widely distributed across very different breeds. For example, h39 was found in breeds as diverse as the Bernese mountain dog, collie, dalmatian, greyhound, poodle, Shetland sheepdog, and West Highland white terrier. The relationship between haplotypes is represented on a network, where haplotypes are separated by mutational steps (assuming that the studied microsatellites evolve in a stepwise manner; Figure 2). This network shows many alternative links between haplotypes, an indication that haplotypes can be related in multiple ways and that homoplasy may have led to the origination of the same haplotype on different occasions. Nevertheless, the network shows that a single breed could contain several haplotypes differing from each other by several mutational steps. For example, haplotypes h72 and h88, present in Shetland sheepdogs, differ at three loci and by up to eight mutational steps (Figure 2). However, the number of Y chromosome haplotypes (mean ± SD = 1.4 ± 0.6) per breed was significantly lower than the number of mtDNA haplotypes (2.6 ± 0.9; Wilcoxon signed rank test, $Z = -3.470$, $P = 0.001$). Regarding individual breeds, 16 had a higher number of mtDNA than Y chromosome haplotypes, whereas only 1 showed the opposite trend. No significant association was found between the variability in the two markers (Spearman rank correlation = 0.461; linear regression, $P > 0.05$).

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TABLE 2

Y chromosome haplotypes (in the text with prefix h) for 314 male dogs representing the 10 groups defined by the Fédération Cynologique Internationale (FCI)

		Haplotype																													
Group	*n* (breeds)	39	31	12	33	17	40	44	71	57	72	75	63	88	82	70	79	18	11	42	34	4	69	73	35	80	7	76	77	78	83
1	65 (17)	29		26	4	1					3			1	1																
2	56 (19)	24	12		3		5	3	2			1				5	1														
3	51 (11)	8	1	15	3	9		2		1	6		1					5													
4 and 6	34 (11)	13			1	6			12										1	1											
5	30 (12)	5	1	1			1		1	5			3								4	3	2	2	1	1					
7	11 (4)	3	1	6						1																					
8	28 (9)	3	13	1								1															6	4			
9	26 (11)	5	1	8	8			1																					1	1	1
10	13 (3)	8	1				4																								

Group 1, sheepdogs and cattle dogs (except Swiss cattle dogs); group 2, pinscher and schnauzer, molossoid breeds, Swiss mountain and cattle dogs and other breeds; group 3, terriers; group 4, dachshunds; group 5, spitz and primitive types; group 6, scenthounds and related breeds; group 7, pointing dogs; group 8, retrievers, flushing dogs, and water dogs; group 9, companion and toy dogs; group 10, sighthounds (http://www.fci.be/nomenclatures.asp?lang=en&sel=0). Groups 4 and 6 are joined because they are considered one single group by some national kennel clubs.

Since the mutational mechanisms and the modes of inheritance are different between the mtDNA and Y chromosome markers, the relevance of the difference between the numbers of haplotypes for each kind of marker in each breed cannot be directly evaluated. Therefore, we have for comparative purposes typed 112 male gray wolves from different populations in Europe and North America (Table 1) for the same markers. The differences in the patterns of diversity between dog breeds and wolf populations can be informative of the differences in the breeding strategies. In these gray wolf populations, contrary to the situation in dogs, the number of Y chromosome haplotypes (6.7 ± 1.4) was significantly larger than the number of mtDNA types (3.5 ± 1.5; Wilcoxon signed rank test, $Z = 2.032$, $P = 0.042$).

The overall number of mtDNA haplotypes observed in the 100 dogs is slightly larger than the total number of Y chromosome types (Table 1). However, the direct comparison with the diversity within the wolf populations does not seem appropriate because over such a long timescale (since the time of domestication) homoplasy is likely to have seriously confounded the results (see Figure 2).

Diversity within groups of dog breeds: We studied patterns of variation in a sample of 314 dogs representing a total of 97 breeds to characterize the diversity observed within and among the 10 groups of dogs recognized by the FCI. Comparison of the Y chromosome haplotypes found in each group showed that while some haplotypes were very common and widely distributed across groups (*i.e.*, h39, h31, h12, and h33; Table 2), many other haplotypes were rare and limited to one or two groups. Of the 30 haplotypes observed, 18 (60%) were observed in only one group and 3 more (10%) in just two groups. There were large differences in diversity among the groups. The highest number of haplotypes (13) and the highest haplotype diversity (0.89) were observed in group 5 (spitz and primitive types, Tables 2 and 3). Of the 13 haplotypes found in this group, 6 (46%) were unique and characterized 43% of the dogs in the group. On the other hand, some well-sampled groups showed much lower diversity. For example, the 65 group 1 dogs (sheepdogs and cattle dogs) from 17 breeds had 7 haplotypes and 2 of those haplotypes were found in 55 (85%) of the dogs. The haplotype diversity of this group (0.63) was among the lowest. Similar to the Y chromosome data, group 5 also showed the largest mtDNA diversity, with a haplotype diversity of 0.96 (Table 3), clearly higher than that for any other group. However, there was no overall correlation between the level of mitochondrial and Y chromosome haplotype diversity in the different groups of dog breeds ($R = 0.285$, $P = 0.46$).

An analysis of the molecular variance indicated that between 9.3% (for the distance based on the stepwise mutation model, $d_{2_{ij}}$) and 10.8% (for the infinite-allele model, $d_{1_{ij}}$) of the Y chromosome diversity was distributed among the different groups. In contrast, only 3.0% of the mtDNA variance was distributed among groups. This implies that the genetic separation among groups of dogs is three or more times larger for males than for females. When these analyses were done ignoring the divergence between haplotypes and considering only the difference in haplotype frequencies between the dog groups, the same results were obtained.

The FCI groups of dog breeds correspond to a functional classification, but may have nothing to do with a genetic classification of dog breeds. Using a larger number of microsatellite markers and single-nucleotide polymorphisms (SNPs), Parker *et al.* (2004) found four groups of dog breeds. We repeated the analyses of the molecular variance for these groupings, with a reduced data

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TABLE 3

Number of haplotypes and haplotype diversity observed at Y chromosome and mtDNA loci for each FCI group of dog breeds

	mtDNA			Y chromosome		
Group	*n*	No. haplotypes	Haplotype diversity	*n*	No. haplotypes	Haplotype diversity
1	41	13	0.874	65	7	0.634
2	38	15	0.885	56	9	0.747
3	12	9	0.833	51	10	0.828
4 and 6	20	8	0.845	34	6	0.696
5	214	50	0.955	30	13	0.891
7	11	6	0.760	11	4	0.612
8	21	4	0.689	28	6	0.704
9	32	13	0.885	26	8	0.766
10	41	20	0.892	13	3	0.521

Groups 4 and 6 are merged into a single group.

set, and the results were basically identical to the results obtained with the FCI groups: about three times more Y chromosome diversity (15.1%) than mtDNA diversity (5.9%) was distributed among the groups of dog breeds.

DISCUSSION

Dog breeds are very well-defined entities and they breed true: the offspring of two dalmatians look like dalmatians, while the offspring of two German shepherds look like German shepherds. This demonstrates that the phenotypic traits that characterize breeds are inherited. Consequently, breeds are expected to be genetically differentiable, and this is what the analysis of autosomal microsatellites has shown. A panel of just 18 markers allowed us to correctly assign almost all dogs belonging to 20 breeds and dogs belonging to the same breed had very similar genotypes. Similar results have been found previously by a number of researchers (Zajc and Sampson 1999; Koskinen 2003; DeNise *et al.* 2004; Parker *et al.* 2004). However, no such clear breed differentiation was observed for mtDNA (see also Vilà *et al.* 1999a; Savolainen *et al.* 2002) or Y chromosome markers. This high frequency of shared haplotypes between breeds may be informative about their origin.

The comparison of levels of diversity in the mtDNA and Y chromosomes of dogs and gray wolves offers a perspective on how the founders of dog breeds may have been selected. Wild gray wolves are essentially monogamous animals living in packs where only the alpha male and the alpha female reproduce every year (Mech 1970; Packard 2003). About as many females as males contribute to reproduction in a single year. Although some differences are expected in the overall contribution by the sexes due to differences in the turnover rate for alpha males and alpha females in wild packs, given that mtDNA and Y chromosomes are haploid, the effective population size of these markers is expected to be similar. Thus, differences in the diversity of Y chromosomes and mtDNA in wolf populations are likely to be the result of differences in selection, rate of mutation, and sex-biased dispersal. The substitution rate for the control region in wolves and coyotes has been estimated to be 5×10^{-8}/bp/generation, which corresponds to a rate of 1.3×10^{-5}/generation for the 261-bp sequence studied here (Vilà *et al.* 1999b). Pedigree analysis of human microsatellites has revealed mutation rates on the order of 10^{-3}–10^{-4} (Ellegren 2000). Since the Y chromosome haplotypes are defined by four microsatellites, we expect a higher mutation rate for this system. Consequently, all else being equal, diversity is expected to be higher for Y chromosome markers, which is observed for wolf populations. Additionally, a possible higher dispersal rate for male than for female wolves (Flagstad *et al.* 2003; however, see Mech and Boitani 2003) could also contribute to greater Y chromosome diversity per population.

Dog breeds were characterized by a pattern of mtDNA and Y chromosome diversity opposite to that found in natural gray wolf populations. Within breeds, fewer Y chromosome haplotypes than mtDNA haplotypes were found. This strongly indicates that a smaller number of males than females were involved in the formation of most breeds. The breeding potential for males is much larger than that for females because a bitch will rarely raise more than one litter per year, while a single male could father a large number of litters. This has resulted in the existence of "popular sires" (Ostrander and Kruglyak 2000), dogs that because of their characteristics have been often used as breeders. Some of these males may produce >100 litters in their lifetime (Ostrander and Kruglyak 2000). Consequently, a phenotypic trait present in one male can be successfully transferred to a larger number of offspring per generation than if that trait is in a female. Therefore, strong selection on males allows a faster definition of the phenotype in a new breed.

Such sex-biased selection may be commonplace among other domestic mammals. A somewhat extreme example of this is the case of the thoroughbred horse. Although

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the breed numbers >300,000 individuals worldwide, an exhaustive pedigree and microsatellite analysis revealed that one single founder stallion was responsible for 95% of the paternal lineages (Cunningham *et al.* 2001). Also, only one Y chromosome lineage has been found in a large number of horse breeds (Lindgren *et al.* 2004) whereas the mtDNA diversity is very large (Vilà *et al.* 2001; Jansen *et al.* 2002). The narrow definition of dog breed standards in recent times (Crowley and Adelman 1998) and the increasing value of purebred dogs strictly fitting the definition (Cunliffe 1999) have led to a strong selective pressure in dogs that promotes this sex bias.

Although the bias toward males in the origin of breeds may be common to all large domestic mammals, in the case of the dog it represents a more extreme deviation from the ancestral social behavior. While the ancestors of other livestock species were presumably polygamous (Houpt and Boyd 1994; Shackleton and The IUCN/SSC Caprine Specialist Group 1997; Nowak 1999; Levine 1999), the gray wolf is essentially monogamous (Mech 1970; Packard 2003). Consequently, both sexes have similar contributions to reproduction on average in gray wolves, so the sex bias observed in dog breeds is the result of a drastic change of the social system of the ancestor.

The comparison of groups of breeds following the classification of the FCI or of the groups derived from the Parker *et al.* (2004) study reveals that groups are more differentiated from each other for Y chromosome than for mtDNA haplotypes. Despite no clear patterns of differentiation across breeds for mtDNA and Y chromosome haplotypes, groups of breeds that share a common function or morphology (corresponding to the FCI groups) are more likely to share Y chromosome than mtDNA haplotypes. This would suggest that when new breeds were formed, male founders were more likely than females to derive from a similar breed from the same group. As a result, a large number of Y chromosome haplotypes were restricted to one or two of the groups (Table 2).

The large diversity in group 5 identified here at both mtDNA and Y chromosome markers supports that this is a very heterogeneous group. Such large diversity could derive from a heterogeneous origin of these primitive dogs from diverse localities or from frequent introgression of wolf DNA through hybridization or backcrosses (Vilà *et al.* 2005). Parker *et al.* (2004) suggested that some breeds from FCI group 5 represent primitive dogs because they occupied an ancestral position in a tree of dog breeds generated using nuclear markers. Similarly, the group including companion and toy dogs (group 9) is also characterized by a large genetic diversity and is represented by breeds from very diverse morphologies and origins.

Data from a large number of autosomal markers led Parker *et al.* (2004) to conclude that most dog breeds have a very recent origin (also see Dennis-Bryan and Clutton-Brock 1988). This view is supported by the lack of unique mtDNA or Y haplotypes within breeds, indicating that breeds have not been isolated for a very long time. This interpretation contrasts with the widespread view that many breeds have very old origins (Crowley and Adelman 1998) on the basis of 4000-year-old representations of dogs with a morphology strikingly similar to that of some modern dog breeds (Clutton-Brock 1999). More than the uninterrupted selection for such dogs in isolation from others for centuries or millennia, these ancient representations may indicate selective breeding for the same phenotype at different points in time (Parker *et al.* 2004). Alternatively, before the establishment of modern breeding strategies, recognizable dog types may have been maintained, but they were far less isolated from each other and from mongrels than they are today.

For thousands of generations, before the advent of modern breeding practices, dogs around the world may have been mating almost "at random." This is supported by the lack of clear patterns in the distribution of mtDNA and Y chromosome haplotypes across the breeds. Despite this, occasionally some morphologically differentiated types of dogs may have existed. From this diverse pool of dogs some individuals were selected as founders and became the seed of new breeds. Due to this recent sorting from an ancient dog gene pool, dogs that belong to the same mtDNA or Y chromosome lineages do not need to be morphologically or behaviorally similar. Selection was centered on males and a bias in the contribution of the sexes may have predominated at the origin of most modern dog breeds.

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LITERATURE CITED

Boitani, L., 2003 Wolf conservation and recovery, pp. 317–340 in *Wolves: Behavior, Ecology, and Conservation*, edited by L. D. Mech and L. Boitani. University of Chicago Press, Chicago.

Clement, M., D. Posada and K. A. Crandall, 2000 TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9:** 1657–1659.

Clutton-Brock, J., 1999 *A Natural History of Domesticated Mammals*, Ed. 2. Cambridge University Press, Cambridge, UK.

Coppinger, R., and L. Coppinger, 2001 *Dogs*. University of Chicago Press, Chicago.

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup and M. Solignac, 1999 New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics **153:** 1989–2000.

Downloaded from https://academic.oup.com/genetics/article/172/2/1121/5923096 by guest on 23 April 2024

Crowley, J., and B. Adelman (Editors), 1998 *The Complete Dog Book; Official Publication of the American Kennel Club*, Ed 19. Howell Book House, New York.

Cunliffe, J., 1999 *The Encyclopedia of Dog Breeds*. Parragon Publishing, Bath, UK.

Cunningham, E. P., J. J. Dooley, R. K. Span and D. G. Bradley, 2001 Microsatellite diversity, pedigree relatedness and the contributions of founder lineages to thoroughbred horses. Anim. Genet. **32:** 360–364.

DeNise, S., E. Johnston, J. Halverson, K. Marshall, D. Rosenfeld *et al.*, 2004 Power of exclusion for parentage verification and probability of match for identity in American kennel club breeds using 17 canine microsatellite markers. Anim. Genet. **35:** 14–17.

Dennis-Bryan, K., and J. Clutton-Brock, 1988 *Dogs of the Last Hundred Years at the British Museum (Natural History)*. British Museum (Natural History), London.

Ellegren, H., 2000 Microsatellite mutations in the germline: implications for evolutionary inference. Trends Genet. **16:** 551–558.

Estoup, A., and J.-M. Cornuet, 1999 Microsatellite evolution: inferences from population data, pp. 49–65 in *Microsatellites: Evolution and Applications*, edited by D. B. Goldstein and C. Schlötterer. Oxford University Press, Oxford.

Excoffier, L., P. Smouse and J. Quattro, 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131:** 479–491.

Falush, D., M. Stephens and J. K. Pritchard, 2003 Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics **164:** 1567–1587.

Flagstad, Ø., C. W. Walker, C. Vilà, A.-K. Sundqvist, B. Fernholm *et al.*, 2003 Two centuries of the Scandinavian wolf population: patterns of genetic variability and migration during an era of dramatic decline. Mol. Ecol. **12:** 869–880.

Francisco, L. V., A. A. Langston, C. S. Mellersh, C. L. Neal and E. A. Ostrander, 1996 A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. Mamm. Genome **7:** 359–362.

Hart, B. L., 1995 Analysing breed and gender differences in behaviour, pp. 65–77 in *The Domestic Dog: Its Evolution, Behaviour and Interactions with People*, edited by J. Serpell. Cambridge University Press, Cambridge, UK.

Houpt, K. A., and L. Boyd, 1994 Social behavior, pp. 229–254 in *Przewalski's Horse: The History and Biology on an Endangered Species*, edited by L. Boyd and K. A. Houpt. State University of New York Press, Albany, NY.

Irion, D. N., A. L. Schaffer, T. R. Famula, M. L. Eggleston, S. S. Hughes *et al.*, 2003 Analysis of genetic variation in 28 dog breed populations with 100 microsatellite markers. J. Hered. **94:** 81–87.

Jansen, T., P. Forster, M. A. Levine, H. Oelke, M. Hurles *et al.*, 2002 Mitochondrial DNA and the origins of the domestic horse. Proc. Natl. Acad. Sci. USA **99:** 10905–10910.

Koskinen, M. T., 2003 Individual assignment using microsatellite DNA reveals unambiguous breed identification in the domestic dog. Anim. Genet. **34:** 297–301.

Leonard, J. A., R. K. Wayne, J. Wheeler, R. Valadez, S. Guillén *et al.*, 2002 Ancient DNA evidence for Old World origin of New World dogs. Science **298:** 1613–1616.

Levine, M. A., 1999 Botai and the origins of horse domestication. J. Anthropol. Archeol. **18:** 29–78.

Lindgren, G., N. Backström, J. Swinburne, L. Hellborg, A. Einarsson *et al.*, 2004 Limited number of patrilines in horse domestication. Nat. Genet. **36:** 335–336.

Mech, L. D., 1970 *The Wolf: The Ecology and Behavior of an Endangered Species*. University of Minnesota Press, Minneapolis.

Mech, L. D., and L. Boitani, 2003 Wolf social ecology, pp. 1–34 in *Wolves: Behavior, Ecology, and Conservation*, edited by L. D. Mech and L. Boitani. University of Chicago Press, Chicago.

Nowak, R. M., 1999 *Walker's Mammals of the World*, Ed. 6. Johns Hopkins University Press, Baltimore.

Okumura, N., N. Ishiguro, M. Nakano, A. Matsui and M. Sahara, 1996 Intra- and interbreed genetic variation of mitochondrial DNA major non-coding regions in Japanese native dog breeds (*Canis familiaris*). Anim. Genet. **27:** 397–405.

Ostrander, E. A., and L. Kruglyak, 2000 Unleashing the canine genome. Genome Res. **10:** 1271–1274.

Ostrander, E. A., G. F. Sprague and J. Rine, 1993 Identification and characterization of dinucleotide repeat $(CA)_n$ markers for genetic-mapping in dog. Genomics **16:** 207–213.

Packard, J. M., 2003 Wolf behavior: reproductive, social and intelligent, pp. 35–65 in *Wolves: Behavior, Ecology, and Conservation*, edited by L. D. Mech and L. Boitani. University of Chicago Press, Chicago.

Parker, H. G., L. V. Kim, N. B. Sutter, S. Carlson, T. D. Lorentzen *et al.*, 2004 Genetic structure of the purebred domestic dog. Science **304:** 1160–1166.

Pritchard, J. K., M. Stephens and P. J. Donnelly, 2000 Inference of population structure using multilocus genotype data. Genetics **155:** 945–959.

Sablin, M. V., and G. A. Khlopachev, 2002 The earliest Ice Age dogs: evidence from Eliseevichi. Curr. Anthropol. **43:** 795–799.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Savolainen, P., Y. P. Zhang, J. Luo, J. Lundeberg and T. Leitner, 2002 Genetic evidence for an East Asian origin of domestic dogs. Science **298:** 1610–1613.

Schwartz, J., 1997 *A History of Dogs in the Early Americas*. Yale University Press, New Haven, CT.

Shackleton, D. M. (Editor), and The IUCN/SSC Caprine Specialist Group, 1997 *Wild Sheep and Goats and their Relatives: Status Survey and Conservation Action Plan for Caprine*. IUCN, Gland, Switzerland/Cambridge, UK.

Shibuya, H., B. K. Collins, T. H. Huang and G. S. Johnson, 1994 A polymorphic $(AGGAAT)_n$ tandem repeat in an intron of canine von Willebrand factor gene. Anim. Genet. **25:** 122.

Sundqvist, A.-K., H. Ellegren, M. Olivier and C. Vilà, 2001 Y chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite markers. Mol. Ecol. **10:** 1959–1966.

Swofford, D. L., 1998 *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4*. Sinauer Associates, Sunderland, MA.

Vilà, C., P. Savolainen, J. E. Maldonado, I. R. Amorim, J. E. Rice *et al.*, 1997 Multiple and ancient origins of the domestic dog. Science **276:** 1687–1689.

Vilà, C., J. Maldonado and R. K. Wayne, 1999a Phylogenetic relationships, evolution, and genetic diversity of the domestic dog. J. Hered. **90:** 71–77.

Vilà, C., I. R. Amorim, J. A. Leonard, D. Posadas, J. Castroviejo *et al.*, 1999b Mitochondrial DNA phylogeography and population history of the gray wolf *Canis lupus*. Mol. Ecol. **8:** 2089–2103.

Vilà, C., J. A. Leonard, A. Götherström, S. Marklund, K. Sandberg *et al.*, 2001 Widespread origins of domestic horse lineages. Science **291:** 474–477.

Vilà, C., J. Seddon and H. Ellegren, 2005 Genes of domestic mammals augmented by backcrossing with wild ancestors. Trends Genet. **21:** 214–218.

Wayne, R. K., 1986a Cranial morphology of domestic and wild canids: the influence of development on morphological change. Evolution **40:** 243–261.

Wayne, R. K., 1986b Limb morphology of domestic and wild canids: the influence of development on morphologic change. J. Morphol. **187:** 301–319.

Wayne, R. K., and C. Vilà, 2001 Phylogeny and origin of the domestic dog, pp. 1–13 in *The Genetics of the Dog*, edited by A. Ruvinsky and J. Sampson. CABI Publishing, New York.

Zajc, I., and J. Sampson, 1999 Utility of canine microsatellites in revealing the relationships of pure bred dogs. J. Hered. **90:** 104–107.

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