The Legacy of Domestication: Accumulation of Deleterious Mutations in the Dog Genome

Fernando Cruz, Carles Vilà,†‡ and Matthew T. Webster§*

*Department of Ecology and Evolution, Biophore, University of Lausanne, Lausanne, Switzerland; †Department of Evolution, Genomics and Systematics, Evolutionary Biology Centre, Uppsala University, Norbyvägen, Uppsala, Sweden; ‡Estación Biológica de Doñana-Consejo Superior de Investigaciones Científicas (CSIC), Avd. María Luisa s/n, 41013 Seville, Spain; and §Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

Dogs exhibit more phenotypic variation than any other mammal and are affected by a wide variety of genetic diseases. However, the origin and genetic basis of this variation is still poorly understood. We examined the effect of domestication on the dog genome by comparison with its wild ancestor, the gray wolf. We compared variation in dog and wolf genes using whole-genome single nucleotide polymorphism (SNP) data. The d_N/d_S ratio (ω) was around 50% greater for SNPs found in dogs than in wolves, indicating that a higher proportion of nonsynonymous alleles segregate in dogs compared with nonfunctional genetic variation. We suggest that the majority of these alleles are slightly deleterious and that two main factors may have contributed to their increase. The first is a relaxation of selective constraint due to a population bottleneck and altered breeding patterns accompanying domestication. The second is a reduction of effective population size at loci linked to those under positive selection due to Hill–Robertson interference. An increase in slightly deleterious genetic variation could contribute to the prevalence of disease in modern dog breeds.

Introduction

Domestic dogs (*Canis familiaris*) have an intimate connection with human society and are valued as workers, hunters, herders, and companions. Recently, their importance has been augmented by their value as a model organism for human disease (Karlsson et al. 2007). Hundreds of genetic disorders have been described in dogs and more than half of them resemble specific human disorders (Ostrander and Kruglyak 2000; Sutter and Ostrander 2004). This is mainly due to the unique evolutionary history of dogs, which can be divided into two main phases (Wayne and Ostrander 2007). The dog was the first mammal to be domesticated, and its relationship with humans began more than 15,000 years ago (Vilà et al. 1997; Sablin and Khlopachev 2002; Lindblad-Toh et al. 2005). The domestication process probably involved the taming of small subsets of the ancestral wolf (*Canis lupus*) from multiple locations. The second phase involved selective breeding, which predominantly occurred in the last few centuries and has resulted in more than 400 recognized breeds (Clutton-Brock 1999). In addition to a variety of genetic diseases, dogs exhibit huge variation in size, shape, physiology, and behavior (Wayne 1986a, 1986b). However, the breeding of dogs was under human control to some extent long before breed creation, imposing a strong selection on characteristics such as behavior for thousands of years. Considering that dogs arose from a small gene pool relatively recently in evolutionary time and that selection for diversity in breeds is even more recent, the huge phenotypic variation observed in modern day breeds is striking.

Evidence is accumulating that domestication in plants and animals causes significant changes in the genome compared with their wild ancestors. Artificial selection has caused selective sweeps at multiple loci in domestic species (Zeder et al. 2006). R. A. Fisher suggested that selection by humans has led to an increase in the number of maladaptive mutations segregating in various dog breeds by genetic hitchhiking (Fisher 1930). Selection at linked sites decreases locus-specific effective population size, which increases the probability of deleterious mutations becoming fixed (Hill and Robertson 1966; Comeron et al. 2008). Population bottlenecks associated with the domestication process could also reduce the efficacy of purifying selection (Kimura 1962).

A comparison of the d_N/d_S ratio (ω) in dog and wolf lineages indicated that dogs appear to have been accumulating nonsynonymous mutations in mitochondrial DNA (mtDNA) genes at a greater rate than wolves (Björnerfeldt et al. 2006). This could be indicative of a general increase in maladaptive mutations in the dog genome. However, there are many ways in which mtDNA may not be representative of the whole genome and the extent to which nuclear genes are affected is unclear. In particular, mtDNA is nonrecombining, maternally inherited, and behaves as a single locus (e.g., Bruford et al. 2003). Furthermore, mtDNA generally exhibits an excess of slightly deleterious mutations segregating in populations compared with divergent sites between species, as observed in *Drosophila*, mice, and hominids (Rand and Kann 1996; Hasegawa et al. 1998). A detailed analysis of the nuclear genome is necessary to understand whether the changes that affected the nonsynonymous diversity in mitochondria had significant impact on the overall functional genetic variation in dogs.

Here we analyze variation in wolf and dog nuclear protein-coding genes on a genomic scale. We use an adaptation of the relative rate test to compare levels of functional genetic variation in wolves and dogs. Our method has the advantage that it compares genetic variation from a common node defined by an outgroup, which provides unbiased estimates of the relative levels of genetic variation in dogs and wolves. We aligned >16 Mb of wolf whole-genome shotgun sequence reads to the dog genome (Lindblad-Toh et al. 2005). We also utilized the whole-genome alignments of the dog and domestic cat (*Felis catus*). We used the cat genome sequence as an outgroup to polarize nucleotide changes along the dog and wolf branches (fig. 1). This enabled us to estimate the relative occurrence of nonsynonymous versus synonymous changes in wolves and dogs.

Key words: genetic drift, bottleneck, selective constraint, purifying selection, domestication, dog genome.

E-mail: matthew.webster@imbim.uu.se.

Mol. Biol. Evol. 25(11):2331–2336. 2008
doi:10.1093/molbev/msn177
Advance Access publication August 9, 2008

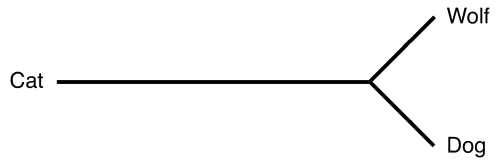

FIG. 1.—Orthologous dog–wolf–cat trio (not to scale). We used maximum likelihood to estimate synonymous and nonsynonymous changes along the dog and wolf branches, using a single sequence from each species.

Materials and Methods

Genomic Sequences

We identified single nucleotide polymorphisms (SNPs) that show different alleles between wolf and dog sequences using alignments of whole-genome shotgun sequences from the gray wolves and the dog genome (canFam2) made using SSAHA-SNP (http://www.sanger.ac.uk/Software/analysis/ssahaSNP/) and presented by Lindblad-Toh et al. (2005). These wolves came from four different geographic areas: Alaska, China, India, and Spain and were each sequenced to 0.004× coverage (~22,000 reads per sample). We obtained SSAHA-SNP output files, which contain high-quality reads that randomly overlap with the dog genome assembly. These reads were trimmed at either end before running SSAHA-SNP, including only blocks longer than 250 bp in which the 20-base running average Phred quality score was at least 20. To be called SNPs, they were required to have a quality score of 23 with a minimum quality score of 15 for each five bases at either side of the SNP and only one mismatch allowed at the flanking 10 bases. The low-coverage 2× assembly of the cat genome (felCat3) was used as an outgroup (Pontius et al. 2007). Chained BlastZ pairwise alignments of the cat and the dog genomes were downloaded from the University of California, Santa Cruz Genome Browser (http://hgdownload.cse.ucsc.edu/downloads.html#dog).

In order to test that our results were not an artifact of sequencing or alignment errors, we repeated our analysis substituting the wolf shotgun sequences with dog shotgun sequences from the same data set (Lindblad-Toh et al. 2005). These sequences came from nine dog breeds with diverse origins, each sequenced to 0.02× coverage (~100,000 reads per sample).

Dog–Wolf–Cat Alignment

We used the above data to make alignments of a single wolf, dog, and cat sequence for all regions of the genome where all three were available. We first constructed pairwise wolf–dog alignments from the SSAHA-SNP output files. The wolf sequences were derived from four individuals, and only one wolf sequence randomly selected was included in the alignment in the case of overlap between more than one wolf sequences. This was done to avoid enrichment of rare wolf alleles in the alignments. However, in practice, wolf reads rarely overlapped (780 bp in total). The cat sequence was then added to the pairwise alignments by identifying the orthologous sequence from the dog–cat whole-genome alignment. The final alignment therefore consisted of a dog sequence derived from the boxer genome sequence, a wolf sequence derived from four individuals, and a cat sequence derived from the cat genome sequence. A summary of the alignment procedure is shown in figure 2.

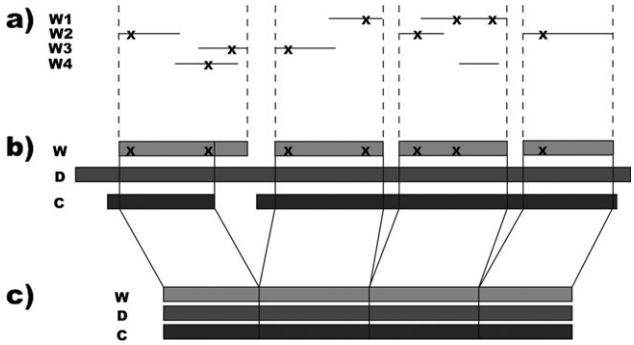

FIG. 2.—Dog–wolf–cat alignment procedure. (*a*) Low-coverage shotgun sequencing reads from four wolf samples (W1–4) were aligned to the dog genome sequence using SSAHA-SNP. (*b*) Wolf reads were combined into a single sequence (W) aligned to the dog genome (D). In the case of overlaps, a single randomly chosen wolf read was used. The dog genome was aligned to the cat genome sequence (C) using BlastZ. (*c*) A single dog-wolf-cat alignment was generated by concatenating all genomic regions where all three sequences were available. This alignment was divided into coding and noncoding sequence using the dog genome annotations.

The dog–wolf–cat alignment was 16.3 Mb long. A total of 14,120 known protein-coding genes identified in canFam2 were downloaded using MartView (http://www.biomart.org/). The coordinates of the dog exons were used to define the coding regions of the alignments, which comprised 241.7 kb (1.5% of the total). These regions were concatenated to form a long alignment consisting of 1,743 total or partial transcripts, removing stop codons and correcting for alternative splicing by avoiding the inclusion of the same codon more than once (for further details, see Appendices 1 and 2 provided as Supplementary Material online). This concatenated coding sequence was 241.2 kb long. A separate alignment of 16.1 Mb was constructed by concatenating the remaining noncoding sequence.

We also constructed a dog–dog–cat alignment, consisting of one dog sequence derived from shotgun sequencing reads, plus dog and cat sequences derived from the full-genome sequences. This alignment was also divided into separate coding and noncoding alignments, which were 3.0 and 180.6 Mb long, respectively. The analyses of noncoding and coding sequences below were also performed on these alignments.

Analysis of Noncoding Sequences

We first assigned SNP alleles in noncoding regions to the lineages leading to wolf or dog using parsimony. When a site differed between the dog and the wolf sequence and one of the alleles matched the cat sequence, the cat base was considered to be the ancestral state. Cases where all three bases differed were ignored (~0.05% of sites). Some differences between the dog and the wolf sequence could represent fixed differences between the dog and the wolf populations. However, many SNPs are shared between

wolves and dogs, which means divergent sites in the alignments can also indicate that the derived allele is likely to be segregating at a higher frequency in the population in which it is inferred to have occurred. We tested for differences in the number of changes inferred on the lineages using a relative rate test, using Tajima's 1*D* method (Tajima 1993). The test allows the calculation of a χ^2 value by using the substitutions of each taxon with respect to the common outgroup and is compared with the χ^2 distribution with one degree of freedom (df). We also counted the number of transition and transversion changes inferred on each lineage.

Analysis of Coding Sequences

The concatenated dog–wolf–cat coding alignment was analyzed using CODEML (PAML v3.15 [Yang 1997]) implementing the codon substitution model of Goldman and Yang (1994) and assuming the F3×4 codon frequency model. This allowed the comparison of the rate of nonsynonymous substitutions (d_N), synonymous substitutions (d_S), and their ratio (d_N/d_S or ω) along different branches of the gene tree. We fitted three models to the alignments. The first model, the one-ratio model, assumes a single ω ratio for all branches in the tree. This was compared using a likelihood ratio test (LRT) with a second model, the two-ratio model. Two separate two-ratio models were fitted: one where the ω ratio was allowed to vary on the dog branch and one where it was allowed to vary on the wolf branch. Finally, the two-ratio model was also compared using an LRT with a third model, the free-ratio model, which assumes an independent ω ratio for each branch.

We used bootstrapping to ensure our results were not affected by biases in the data set. We resampled the data set with replacement, using alignment segments derived from individual wolf transcripts as units. Each of 1,000 bootstrap replicates was then analyzed with CODEML under the one-ratio model and compared with a two-ratio model where the dog branch was allowed to vary. We counted the number of cases where the two-ratio model showed a higher likelihood than the one-ratio model and where ω for the dog branch was greater than the wolf branch.

Radical and Conservative Amino Acid Changes

We tested for differences in occurrence of amino acid changes of small and large effects in both canids by comparing their sequence to the ancestral sequence inferred by maximum likelihood, using the polarity and volume criteria in Zhang (2000). The total numbers of radical and conservative amino acid changes on the wolf and dog lineages were compared using a Fisher's exact test.

Results

Higher Divergence in Shotgun Sequencing Reads

We estimated the number of sites that differed from the ancestral site in the wolf and the dog genomes by analyzing 16.1 Mb of noncoding DNA in the dog–wolf–cat alignment (see figure 1) by maximum parsimony. Although the wolf sequence showed 12,362 divergent sites, the dog had only 10,639 changes (table 1). A relative rate test indicates that these differences are highly significant ($\chi^2 = 129$, $P < 0.001$). When this analysis was repeated using the dog–dog–cat alignment, we inferred 92,055 divergent sites in the sequence derived from dog shotgun reads, compared with 75,350 in the dog genome sequence ($\chi^2 = 1{,}667$, $P < 0.001$). This result shows that the sequences derived from low-coverage whole-genome shotgun reads tend to be more diverged from the inferred ancestral sequence.

Table 1
Nucleotide Changes in Noncoding Regions

Branch	Transitions	Transversions	Total	Transition Bias
Dog–dog–cat alignment				
Dog shotgun	65,279	26,776	92,055	2.44
Dog genome	55,632	19,718	75,350	2.82
Dog–wolf–cat alignment				
Wolf shotgun	8,713	3,649	12,362	2.39
Dog genome	7,805	2,834	10,639	2.75

Table 1 also shows that the nucleotide changes on the dog genome branch in both alignments have a higher transition bias than those inferred from shotgun reads. This difference is significant for both alignments using Fisher's exact test (dog–dog–cat, $P < 2.2 \times 10^{-16}$; dog–wolf–cat, $P = 1.3 \times 10^{-6}$). Patterns of nucleotide substitution in mammalian genes typically exhibit transition biases of three–five (Rosenberg et al. 2003). The changes inferred to occur on the dog genome branch are closer to this expectation. We therefore find a larger number of nucleotide changes and lower transition bias on the branches leading to sequences derived from shotgun sequencing reads. These findings are most readily explained by the presence of a higher proportion of sequencing errors in the shotgun sequences compared with the dog genome sequence or by the alignment of the shotgun sequences to nonorthologous regions of the dog genome by SSAHA-SNP. Nucleotide changes inferred along the branch leading to the dog genome sequence are therefore likely to contain a lower proportion of errors.

Higher Proportion of Nonsynonymous Alleles in Dog

To compare the levels of natural selection acting on the codon changes of the wild and the domestic canid, we used information from the genomic alignment of 80,385 codons between a dog, wolf, and cat sequence. The contribution of the four wolf samples from China, Alaska, India, and Spain was roughly similar, accounting for 26%, 28%, 27%, and 18% of the total alignment length, respectively. First, we fitted three different evolutionary branch models to the alignment to analyze how ω varies among the dog, wolf, and cat lineages in the tree (ω_D, ω_W, and ω_C; table 2). The comparison of the one-ratio model to the two-ratio model (allowing a free estimation of ω_D), using an LRT, showed that the two-ratio model was a significantly better fit than the one-ratio ($2\Delta\ln L = 3.876$; df = 1; $P < 0.05$). Under the two-ratio model,

Table 2
Values of ω under Different Evolutionary Models

Branch model	*np*	ln*L*	ω_D	ω_W	ω_C
One-ratio	6	−421071.3	0.2103	0.2103	0.2103
Two-ratio$_D$	7	−421069.4	0.3225	0.2098	0.2098
Two-ratio$_W$	7	−421071.3	0.2103	0.2179	0.2103
Free-ratio	9	−421069.2	0.3252	0.2020	0.2214

NOTE.—Values of ω (d_N/d_S) estimated in 241 kb of coding DNA (known genes). Where *np* is the number of parameters and *lnL* the logarithm of the likelihood estimated in CODEML for each evolutionary branch model. Two-ratio$_D$ and two-ratio$_W$ refer to a two-ratio model where the dog and wolf branches, respectively, were allowed to vary from the rest of the tree. ω_D, ω_W, and ω_C are the estimated ω for dog, wolf, and cat, respectively.

Table 3
Proportion of Nonsynonymous and Synonymous Changes

Species	ω	N·d_N	S·d_S
Dog	0.3225	49.3	58.1
	(0.209–0.5121)	(32.4–69.2)	(37.7–78.6)
Wolf	0.2098	45.7	82.8
	(0.1947–0.2265)	(34.6–57.9)	(63.6–103.8)
Cat	0.2098	5515.6	9989.3
	(0.1947–0.2265)	(3644.6–6035.7)	(6658.6–10711.9)

NOTE.—Values of ω (d_N/d_S), the absolute number of nonsynonymous (N·d_N), and synonymous changes (S·d_S) estimated by maximum likelihood for both canine genomes and their outgroup. Parameters were estimated under the two-ratio model in CODEML with bootstrap 1,000 replicates. The brackets indicate the 95% bootstrap confidence intervals. Bootstrapping units are transcripts or partial transcript in the dog–wolf–cat alignment from the known genes data set.

ω_D was 53.7% higher than ω_W. The free-ratio model is not a significantly better fit than the two-ratio model ($2\Delta\ln L = 0.412$; df = 2; not significant [n.s.]). However, the estimated ω for each branch are consistent between the two models, with an elevated ω_D (table 2). We repeated the analysis by comparing a two-ratio model where ω_W was allowed to vary with the one-ratio model. Here, an LRT showed no significant differences ($2\Delta\ln L = 0.0304$; df = 1; n.s.), and ω_W was 0.2103 and 0.2179 for the one-ratio and two-ratio models, respectively.

The dog–wolf–cat alignment is a concatenation of codons from 1,743 different transcripts. We bootstrapped this alignment 1,000 times, by randomly choosing 1,743 transcripts with replacement. Each bootstrap replicate was fitted to the one-ratio and two-ratio models by maximum likelihood. The likelihood of the two-ratio model was higher than the likelihood of the one-ratio model with $\omega_D > \omega_W$ in 97.4% of occasions.

Table 3 shows the estimated values of ω for all branches under the two-ratio model, which was the best fit. Also shown are the estimated total numbers of synonymous and nonsynonymous changes. The numbers of nonsynonymous changes on the dog and wolf branches are similar, but the wolf branch has an elevated number of synonymous changes, which results in a higher proportion of nonsynonymous SNPs in the dog population. This is in agreement with the higher diversity observed in the wolf population at noncoding sites.

In order to control for the potential effect of sequencing errors, we repeated part of the above analysis using the dog–dog–cat alignment, which contained a total of 995,179 codons. In this case, the most supported model was the free-ratio model, where ω_S (estimated in the dog shotgun-derived sequence branch) was 0.279, ω_D (estimated in the dog genome-derived sequence branch) was 0.222, and ω_C (estimated in the cat branch using the cat genome sequence) was 0.203. This model was significantly better than the two-ratio model where the dog branch was allowed to vary ($2\Delta\ln L = 30.126$; df = 2; $P < 0.001$) that in turn was significantly better than the one-ratio model ($2\Delta\ln L = 5.945$; df = 1; $P < 0.05$). We also repeated this analysis taking shotgun reads from each of the nine different breeds separately (for details of breeds, see Lindblad-Toh et al. [2005]). Using the free-ratio model, we observed a higher ω on the dog shotgun-derived branch in every case (data not shown).

We estimated the functional impact of the amino acid changes in wolf and dog by categorizing them as radical or conservative. All changes with respect to the ancestral sequence reconstructed by maximum likelihood were taken into account in this analysis using the polarity and volume criteria described by Zhang (2000). There were 27 radical differences and 17 conservative changes in wolf, whereas in dog, these numbers were 22 and 27, respectively. There is therefore an excess of conservative changes in dog although the difference is not significant (two-tail Fisher's exact test; $P = 0.146$).

Discussion

We find evidence of an increased accumulation of nonsynonymous mutations in the dog genome since domestication. Below we argue that the excess nonsynonymous changes in dogs are likely to be mainly slightly deleterious and that two major factors may have influenced their accumulation. The first is a relaxation of selective constraint, which could be due to either demographic factors or changes in the selective regime concurrent with domestication (Björnerfeldt et al. 2006). The second factor is the effect of positive selection at linked sites, which reduces the probability that slightly deleterious mutations will be purged from the population, due to Hill–Robertson interference (Hill and Robertson 1966).

It is important to consider the potential contribution of sequencing or alignment errors to our results. We compared wolf sequences, consisting of short, low-coverage shotgun reads, with the dog and cat genome assemblies, which consist of high-quality contigs. Our analysis shows that the wolf sequences are likely to have a larger proportion of sequencing errors and be more frequently aligned to nonorthologous regions of the dog and cat genomes. Errors such as these would be expected to increase our estimate of ω along the wolf lineage. This is because random changes would affect the nonsynonymous and synonymous rates equally, which would cause ω to become closer to one. However, as we estimate that ω is significantly higher in dogs compared with wolves, our results cannot be explained by sequencing and/or alignment errors.

Dogs have a complex demographic history involving several contractions in effective population size (N_e). Population bottlenecks occurred during domestication from

ancestral wolf population (Vilà et al. 1997; Savolainen et al. 2002) and were associated with prehistoric and recent human population changes and migrations. Severe bottlenecks also occurred during breed creation in the last few hundred years (Wayne and Ostrander 2007). In addition to demographic effects, the domestication of dogs resulted in their breeding behavior coming under the influence of humans. Early dogs were likely selected for traits such as tameness and the ability to bark (Saetre et al. 2004), whereas selection for a variety of specialized attributes and behaviors occurred during breed formation. Both demographic effects and human control of breeding could lead to a relaxation of purifying selection across the dog genome, resulting in slightly deleterious genetic variants increasing in frequency.

An increase in slightly deleterious genetic variation can also result from Hill–Robertson interference (Hill and Robertson 1966). This occurs when linkage between sites under natural selection reduces the overall efficacy of selection. When a particular locus is evolving under the influence of selection, local Hill–Robertson effects reduce the locus-specific N_e at neighboring loci (Comeron et al. 2008). Humans selected for a variety of traits throughout dog evolution, most notably during breed creation, when major effect alleles were fixed in particular breeds (e.g., Leegwater et al. 2007; Sutter et al. 2007). It is therefore possible that strong artificial selection at multiple loci led to a concurrent reduction in the efficacy of purifying selection at loci across the dog genome.

The domestication of dogs and breed creation is likely to have led to reductions in N_e, both at specific loci, due to the effects of linked selection, and across the genome, due to the population bottlenecks. Figure 3 shows the effect of changing N_e on the probability of fixation of a slightly deleterious allele relative to a neutral allele, estimated from Kimura's diffusion approximation (Kimura 1962). Alleles with a higher probability of fixation are predicted to rise in frequency in a population. When N_e is 10,000 and the selective coefficient (s) is close to $1/2N_e$, the probability of fixation is about one-third that of a neutral allele. A halving of N_e leads to nearly a doubling of this probability. It is conceivable that the formation of domestic dog breeds entailed even more drastic reductions in effective population size than this (Vilà et al. 1997), particularly at loci close to those under strong selection. This would have led to an increase in fixation probability for many weakly deleterious alleles.

Several specific loci have now been identified that have been under selection by humans to produce domestic species with traits valued by human society, such as those controlling branching and seed morphology in maize (Zeder et al. 2006) and muscularity in domestic pigs (Andersson and Georges 2004). However, artificial selection for desirable traits in domestic species may also entail a "cost" of accumulation of deleterious alleles. This is indicated by an increased proportion of nonsynonymous genetic variation in rice strains (Lu et al. 2006). Accumulation of deleterious variants has also been proposed in the dog mtDNA genome (Björnerfeldt et al. 2006). Future studies are necessary to determine whether this is a general consequence of domestication.

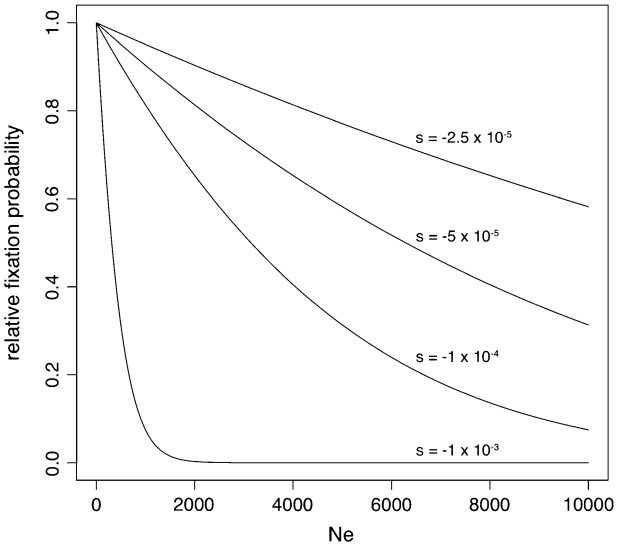

FIG. 3.—The effect of effective population size on the fixation probability of weakly deleterious alleles. Each line shows the relative probability of fixation of a new deleterious allele (selective coefficients shown) relative to the probability of fixation of a neutral allele. The probability of fixation of neutral and slightly deleterious is similar at low effective population sizes.

Conclusions

Here we present evidence for a general trend for nonsynonymous mutations to have increased in frequency in dogs. This indicates that the domestication process has led to an increase in functional genetic variation. Apart from nonsynonymous changes in proteins, several additional sources of variation have been discovered in dogs. High levels of tandem repeat variation have been found in coding regions of genes that influence polydactyly and skull shape (Fondon and Garner 2004). Moreover, polymorphic short interspersed elements in promoter regions are believed to contribute to variation in gene expression (Wang and Kirkness 2005). It is likely that the increase in functional genetic variation is not confined to amino acid changes and that changes in noncoding gene regulatory elements, repeat insertions, and rearrangements are all likely to play an important role in generating dog phenotypic diversity. This excess of functional genetic variation emphasizes the important role of the dog in disease mapping studies.

Supplementary Material

Appendices 1 and 2 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

This research was supported by a postdoctoral scholarship from "Stiftelsen Wenner-Grenska Samfundet" (F.C.), the Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning (C.V.), and the Swedish Research Council (M.T.W.). We thank Claire

Wade for providing dog SNP data and for helpful comments. We thank Greger Larson and Kerstin Lindblad-Toh for critical reading of the manuscript. We thank John McDonald and three anonymous referees for comments that considerably improved the manuscript.

Literature Cited

Andersson L, Georges M. 2004. Domestic-animal genomics: deciphering the genetics of complex traits. Nat Rev Genet. 5:202–212.

Björnerfeldt S, Webster MT, Vilà C. 2006. Relaxation of selective constraint on dog mitochondrial DNA following domestication. Genome Res. 16:990–994.

Bruford MW, Bradley DG, Luikart G. 2003. DNA markers reveal the complexity of livestock domestication. Nat Rev Genet. 4:900.

Clutton-Brock J. 1999. A natural history of domesticated mammals. Cambridge: Cambridge University Press.

Comeron JM, Williford A, Kliman RM. 2008. The Hill-Robertson effect: evolutionary consequences of weak selection and linkage in finite populations. Heredity. 100:19–31.

Fisher RA. 1930. The genetical theory of natural selection. Oxford: Oxford University Press.

Fondon JW III, Garner HR. 2004. Molecular origins of rapid and continuous morphological evolution. Proc Natl Acad Sci USA. 101:18058–18063.

Goldman N, Yang Z. 1994. A codon-based model of nucleotide substitution for protein-coding DNA sequences. Mol Biol Evol. 11:725–736.

Hasegawa M, Cao Y, Yang Z. 1998. Preponderance of slightly deleterious polymorphism in mitochondrial DNA: nonsynonymous/synonymous rate ratio is much higher within species than between species. Mol Biol Evol. 15:1499–1505.

Hill WG, Robertson A. 1966. The effect of linkage on limits to artificial selection. Genet Res. 8:269–294.

Karlsson EK, Baranowska I, Wade CM, et al. (20 co-authors). 2007. Efficient mapping of mendelian traits in dogs through genome-wide association. Nat Genet. 39:1321–1328.

Kimura M. 1962. On the probability of fixation of mutant genes in a population. Genetics. 47:713–719.

Leegwater PA, van Hagen MA, van Oost BA. 2007. Localization of white spotting locus in Boxer dogs on CFA20 by genome-wide linkage analysis with 1500 SNPs. J Hered. 98:549–552.

Lindblad-Toh K, Wade CM, Mikkelsen TS, et al. (46 co-authors). 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 438:803.

Lu J, Tang T, Tang H, Huang J, Shi S, Wu C-I. 2006. The accumulation of deleterious mutations in rice genomes: a hypothesis on the cost of domestication. Trends Genet. 22:126–131.

Ostrander EA, Kruglyak L. 2000. Unleashing the canine genome. Genome Res. 10:1271–1274.

Pontius JU, Mullikin JC, Smith DR, et al. (24 co-authors). 2007. Initial sequence and comparative analysis of the cat genome. Genome Res. 17:1675–1689.

Rand DM, Kann LM. 1996. Excess amino acid polymorphism in mitochondrial DNA: contrasts among genes from Drosophila, mice, and humans. Mol Biol Evol. 13:735–748.

Rosenberg MS, Subramanian S, Kumar S. 2003. Patterns of transitional mutation biases within and among mammalian genomes. Mol Biol Evol. 20:988–993.

Sablin MV, Khlopachev GA. 2002. The earliest ice age dogs: evidence from Eliseevichi 1. Curr Anthropol. 43:795–799.

Saetre P, Lindberg J, Leonard JA, Olsson K, Pettersson U, Ellegren H, Bergstrom TF, Vilà C, Jazin E. 2004. From wild wolf to domestic dog: gene expression changes in the brain. Mol Brain Res. 126:198–206.

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

Sutter NB, Bustamante CD, Chase K, et al. (21 co-authors). 2007. A single IGF1 allele is a major determinant of small size in dogs. Science. 316:112–115.

Sutter NB, Ostrander EA. 2004. Dog star rising: the canine genetic system. Nat Rev Genet. 5:900–910.

Tajima F. 1993. Simple methods for testing the molecular evolutionary clock hypothesis. Genetics. 135:599–607.

Vilà C, Savolainen P, Maldonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK. 1997. Multiple and ancient origins of the domestic dog. Science. 276:1687–1689.

Wang W, Kirkness EF. 2005. Short interspersed elements (SINEs) are a major source of canine genomic diversity. Genome Res. 15:1798–1808.

Wayne RK. 1986a. Limb morphology of domestic and wild canids: the influence of development on morphologic change. J Morphol. 187:301–319.

Wayne RK. 1986b. Cranial morphology of domestic and wild canids: the influence of development on morphological change. Evolution. 40:243.

Wayne RK, Ostrander EA. 2007. Lessons learned from the dog genome. Trends Genet. 23:557–567.

Yang Z. 1997. PAML: a program for package for phylogenetic analysis by maximum likelihood. Comput Appl Biosci. 13:555–556.

Zeder MA, Emshwiller E, Smith BD, Bradley DG. 2006. Documenting domestication: the intersection of genetics and archaeology. Trends Genet. 22:139–155.

Zhang J. 2000. Rates of conservative and radical nonsynonymous nucleotide substitutions in mammalian nuclear genes. J Mol Evol. 50:56–68.

John H McDonald, Associate Editor

Accepted August 1, 2008