

1 **Linkage Disequilibrium and Demographic History of Wild and Domestic Canids**

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ABSTRACT

Assessing the extent of linkage disequilibrium (LD) in natural populations of a non-model species has been difficult due to the lack of available genomic markers. However, with advances in genotyping and genome sequencing, genomic characterization of natural populations has become feasible. Using sequence data and SNP genotypes, we measured linkage disequilibrium (LD) and modeled the demographic history of wild canid populations and domestic dog breeds. In 11 gray wolf and one coyote population, we find that the extent of LD as measured by the distance at which r^2 equals 0.2, extends <10kb in outbred populations to over 1.7Mb in populations that have experienced significant founder events and bottlenecks. This large range in the extent of LD parallels that observed in 18 dog breeds where the r^2 value varies from ~20kb to >5Mb. Furthermore, in modeling demographic history under a composite likelihood framework, we find that two of five wild canid populations exhibit evidence of a historical population contraction. Five domestic dog breeds display evidence for a minor population contraction during domestication, and a more severe contraction during breed formation. Only a 5% reduction in nucleotide diversity was observed as a result of domestication, whereas the loss of nucleotide diversity with breed formation averaged 35%.

47 INTRODUCTION

48 Recombination, recurrent mutation, selection, admixture, and mate choice are factors that
49 influence the extent of linkage disequilibrium (LD) within a species (DEONIER *et al.* 2005; GAUT
50 and LONG 2003; MUELLER 2004). The extent of LD is one of several attributes that affects how
51 readily phenotypic traits in natural populations can be mapped using whole genome association
52 studies. Extensive LD allows associations to be detected more readily using a small number of
53 distantly placed informative markers, whereas low LD necessitates fine-scale mapping (KOHN *et al.*
54 *et al.* 2006; SLATE 2005; STEINER *et al.* 2007). Several studies have measured the extent of LD in
55 plant, invertebrate and domestic vertebrate species (CUTTER *et al.* 2006; FARNIR *et al.* 2000;
56 HADDRILL *et al.* 2005; HARMEGNIES *et al.* 2006; INGVARSSON 2005; MCRAE *et al.* 2002;
57 REMINGTON *et al.* 2001); however, little is known about the extent of LD in wild populations of
58 non-human vertebrates.

59 Among populations of the same species that share similar rates of recombination and
60 mutation and where selection is weak, a critical variable for determining the extent of LD is
61 demographic history. In general, populations which have remained large for a substantial period
62 of time or have rapidly expanded demonstrate lower levels of LD than those that are small or
63 have experienced recent population bottlenecks (GAUT and LONG 2003; MUELLER 2004;
64 PRITCHARD and PRZEWORSKI 2001; REICH *et al.* 2001). Therefore, the extent of LD can be used
65 as a tool to infer demographic history. With the exception of a few model species, the extent of
66 LD has not been used to explore population history primarily because large numbers of markers
67 need to be typed in a substantial number of samples. To date, there have been only a few wild
68 vertebrate species for which the extent of LD has been carefully documented: the collared
69 flycatcher (*Ficedula albicollis*) (BACKSTROM *et al.* 2006), red deer (*Cervus elaphus*) (SLATE and

70 PEMBERTON 2007), and wild mice (*Mus musculus domesticus*) (LAURIE *et al.* 2007).
71 Additionally, LD was modeled in a wild Soay Sheep population using simulations that included
72 parameters of population history (MCRAE *et al.* 2005).

73 The domestic dog (*Canis familiaris*) is emerging as an important model for understanding
74 the genetic basis of morphology, behavior, and disease in mammals (OSTRANDER and WAYNE
75 2005; PARKER and OSTRANDER 2005; SUTTER and OSTRANDER 2004; WAYNE and OSTRANDER
76 2007). In 2005, a 7.8x whole-genome shotgun sequence and assembly of the Boxer was
77 completed (LINDBLAD-TOH *et al.* 2005). In addition, a 1.5x survey sequence of the Standard
78 Poodle became publically available in 2003 (KIRKNESS *et al.* 2003). These two resources,
79 together with 100,000 random sequence reads from nine other dogs of unrelated breeds and
80 20,000 sequence reads from each of four gray wolves (*Canis lupus*) and one coyote (*Canis*
81 *latrans*) (LINDBLAD-TOH *et al.* 2005), provide extensive resources for identifying markers for
82 large scale genetic analysis of wild canid species.

83 In this paper, we utilize dog-derived single nucleotide polymorphisms (SNPs) as well as
84 extensive resequencing to obtain estimates of LD in wild and domestic canids. Sutter *et al.*
85 (2004) first characterized the extent of LD in five dog breeds across five 1Mb regions, followed
86 by Linbladh-toh *et al.* (2005), who examined the extent of LD within 10 dog breeds across a 15
87 Mb region. We expand the number of domestic dog breeds for which the extent of LD is
88 estimated (12 new breeds), although our primary goal is to determine the range that LD extends
89 in a large panel of wild canid populations, including gray wolves and coyotes, and compare these
90 estimates to those from domestic dog breeds. Furthermore, we explore the relationship of LD and
91 demographic history by comparing estimates of LD to known population histories. We also
92 model population histories using the site frequency spectra (SFS) of each population based on

93 origination from an ancestral wolf population followed by specific demographic scenarios. These
94 predicted SFS are then compared to those observed.

95 METHODS

96 Blood, tissue, or buccal swab samples were collected from 908 individuals: 18 dog
97 breeds, $n=546$ (unrelated at the grandparent level); 14 gray wolf populations, $n=344$; and one
98 coyote population, $n=18$ (Table 1). To determine the rate of successful amplification of dog
99 derived molecular markers in distant relatives of the domestic dog, an additional 93 samples were
100 typed from golden jackal (*Canis aureus*), bat-eared fox (*Otocyon megalotis*), gray fox (*Urocyon*
101 *cinareoargenteus*) and Channel Island fox (*Urocyon littoralis*). For samples with low DNA
102 concentrations, whole genome amplification was performed according to manufacturer
103 guidelines (Qiagen REPLI-g kit; QIAGEN INC., VALENCIA, CA).

104 Gray wolf populations sampled varied in demographic history, and include individuals
105 from large outbred populations and from smaller inbred or recently bottlenecked populations
106 (Table S1). Furthermore, the populations chosen here have been the focus of previous genetic
107 research (LEHMAN *et al.* 1992; LEHMAN and WAYNE 1991; LEONARD *et al.* 2005; MUSIANI *et al.*
108 2007; RAMIREZ *et al.* 2006; ROY *et al.* 1994; ROY *et al.* 1996; VILÀ *et al.* 1999a) such that
109 demographic and population genetic conclusions from LD patterns can be independently
110 verified.

111 The domestic dog breeds included in this study also vary in relatedness and demographic
112 history; thus, they provide a test of the use of LD to assess population demography across a
113 variety of timescales and population sizes. American Kennel Club (AKC) registration statistics
114 (AKC website: http://www.akc.org/reg/dogreg_stats.cfm) were used as a proxy for effective
115 population size and recent demographic history. Kendall's tau and a Mantel's test were

116 performed to determine the significance of association between LD and the log of the number of
117 registered individuals.

118 All 1001 samples were genotyped on an ABI 3730 (APPLIED BIOSYSTEMS INC., FOSTER
119 CITY, CA) for 106 SNP loci (Table 1; Figure S1) using a custom set of primers designed for the
120 SNPlex genotyping system (APPLIED BIOSYSTEMS INC., FOSTER CITY, CA). The 106 SNPs
121 genotyped were chosen as a representative subset of the 200 SNPs described previously in Sutter
122 et al. (2004), which were ascertained by direct resequencing of 5 loci on 5 chromosomes each
123 spanning a non-contiguous 5Mb region. Genemapper 4.0 was used to make genotype calls for
124 each SNP locus (APPLIED BIOSYSTEMS INC., FOSTER CITY, CA).

125 To determine the effect of ascertainment bias on LD estimates from genotype data and to
126 model demographic history (see below), sequencing of 18 amplicons spaced across a non-
127 contiguous 5Mb region of chromosome 1 (similar to Sutter et al. 2004) was performed on 188
128 individuals (a subset of genotyped individuals): 5 breeds of dog, $n=97$ (same as Sutter et al.); 4
129 gray wolf populations, $n=73$; a coyote population, $n=17$; and one golden jackal (Table S2 &
130 Figure S2). Eleven of the 18 amplicons were reported previously in Sutter et al. (2004). The 11
131 amplicons were chosen to minimize the amount of sequencing but still measure low to medium
132 range LD (i.e. 10-100kb). We designed and sequenced an additional seven amplicons, spaced at
133 50kb intervals from the central region on chromosome 1, to enhance this latter goal (Figure S2 &
134 Table S2). Sequences were run on an ABI 3730 and polymorphisms were identified and viewed
135 using Phred/Phrap/Consed/Polyphred (EWING and GREEN 1998; EWING *et al.* 1998; GORDON *et*
136 *al.* 1998; NICKERSON *et al.* 1997). All data will be made available on upon request.

137 **Analysis**

138 GENEPOP (RAYMOND and ROUSSET 1995) was used to calculate levels of heterozygosity,
139 and Hardy-Weinberg equilibrium. Estimates of nucleotide diversity were calculated from
140 sequence data representing a sampling of one chromosome per individual across 2000 iterations.
141 This was done to account for inbreeding within breeds and potential sampling of closely related
142 individuals in wild populations. To determine if and to what degree there was a loss of diversity
143 during the domestication of dogs, we sampled one chromosome from each of five domestic dog
144 breeds and four wild canid populations and calculated loss of diversity as $1 - (\pi \text{ in dogs}) / (\pi \text{ in}$
145 $\text{wolves})$. Additionally, the loss of nucleotide diversity at breed formation ($1 - (\pi \text{ in breed}) / (\pi \text{ in}$
146 $\text{dogs})$) was calculated by sampling one chromosome from each individual of each breed of dog
147 and one chromosome from each individual across all breeds. Haplotypes were inferred across
148 each population using the software program PHASE (STEPHENS and DONNELLY 2003; STEPHENS
149 *et al.* 2001). The percent of haplotypes within and among breeds/populations were calculated as
150 in Sutter *et al.* (2004). The software program Haploview (BARRETT *et al.* 2005) was used to
151 calculate r^2 and D' , which were plotted by matching allele frequencies between pairs of SNPs
152 with an allele frequency difference of $<10\%$ (EBERLE *et al.* 2006). Median values for each
153 distance category were calculated and a logarithmic curve was fitted to the data. An r^2 of 0.2 was
154 arbitrarily chosen as the value for which the extent of LD was compared between populations
155 and species.

156 **Ascertainment Bias:**

157 The discovery panel for genotyped loci contained 5 breeds of dog: one breed from each
158 of 5 distinct phylogenetic groupings (PARKER *et al.* 2004; PARKER *et al.* 2007; SUTTER *et al.*
159 2004). Genotyping these loci in populations outside the discovery panel will likely result in
160 ascertainment bias because SNPs at high frequency in one population/breed may be rare or

161 absent in others. Thus, the level of ascertainment bias present in this study was assessed by
162 performing simulations that replicated the ascertainment scheme (Figure S3). This scheme
163 utilized the original sequence data (SUTTER *et al.* 2004) in the following procedure: (1) one breed
164 of dog was randomly chosen as the focus breed and individuals from the 4 other breeds were
165 designated as the ascertainment panel; (2) For each amplicon, two individuals were randomly
166 selected from the ascertainment panel and compared. If any marker was segregating, it was
167 flagged as a SNP and “genotyped” in the focus breed. This sampling was repeated for 2000
168 bootstraps; (3) the extent of LD was calculated for the focus breed and compared to the extent of
169 LD based on the observed sequence data. This sampling increased the diversity of sequence
170 comparisons used and accounted for possible unobserved sequences due to recombination among
171 amplicons. Also, we wanted to reduce the bias caused by using a limited set of starting points.

172 **Population Structure:**

173 Principal component analysis (PCA) using the program EIGENSTRAT (PRICE *et al.* 2006)
174 was performed on genotyped loci to determine if the 106 SNP dataset identified population
175 substructure. Twstats (PATTERSON *et al.* 2006) in EIGENSTRAT , was used to determine the
176 number of significant principal components. As “a rule of thumb,” one plus the number of
177 significant principal components is considered the number of groups identified by the PCA.

178 **Demographic Modeling:**

179 To explore the demographic history of wild canid populations and domestic dog breeds,
180 we used the program PRFREQ (BOYKO *et al.* 2008; WILLIAMSON *et al.* 2005) to estimate
181 demographic parameters under a composite likelihood framework. The program utilizes the
182 Poisson Random Field (PRF) approach (SAWYER and HARTL 1992), which predicts the
183 distribution of allele frequencies across sites based on single-locus diffusion theory.

184 Demographic parameters are then estimated by maximum likelihood using the site frequency
185 spectra (SFS). Assumptions of the program are the Wright-Fisher model of mutation and
186 independence among sites. However, the majority of loci utilized in this study were in linkage
187 disequilibrium. Therefore, the likelihoods should be interpreted as a “composite-likelihood”
188 function, an approximation of the true likelihood (CAICEDO *et al.* 2007), as the assumption of
189 independence between sites is violated. Because of the violation of linkage equilibrium,
190 simulations of linked sites within amplicons were performed to verify our p-values (see
191 supplemental text). Two likelihood functions were used to make inferences. The first is based on
192 the number of SNPs in each frequency class (denoted "Poisson"), and the second is based on the
193 proportion of SNPs in each class (denoted "multinomial"). The Poisson likelihood function is
194 much more powerful for inference of bottlenecks, since it takes into account the degree of
195 reduction in diversity as well as the skew in allele frequency distribution. The multinomial
196 likelihood function captures only the latter, but has the advantage of not requiring *a priori*
197 assumptions regarding the population-scaled mutation rate, θ . Across models, the significance of
198 incorporating additional demographic parameters was assessed by using the likelihood ratio test
199 ($2\log[L(\text{model 1})/L(\text{model 2})]$).

200 **Domestication Model:**

201 To model the initial domestication event in dogs (Figure 1a), sequence data from Sutter et
202 al. (2004) derived from five breeds of dog were combined with genotype data from this study,
203 producing a combined dataset containing 22 dog breeds. Loci common to both datasets and not
204 segregating in the outgroup (golden jackal) were retained for further analysis (82 SNPs). To
205 generate an unfolded site frequency spectrum representative of the ancestral domesticated dog
206 population to estimate demographic parameters, we randomly selected one chromosome from

207 each breed for 2000 iterations and constructed a SFS averaged across iterations. SNPs with
208 sample sizes less than 14 (due to missing data) were excluded, leaving a total of 76 SNPs. SNPs
209 with sample sizes greater than 14 were “projected” to a sample size of 14. This involved using
210 the hypergeometric distribution to calculate the probability of the latter falling into each
211 frequency class of a SFS with sample size 14, and summing over all SNPs in each frequency
212 class to create the final SFS (CLARK *et al.* 2005).

213 To control for the effect of ascertainment bias on our observed SFS, we chose to create a
214 corrected site frequency spectrum as outlined in Nielsen *et al.* (2004). Corrections were made
215 under the basic model, assuming all SNPs were ascertained at a discovery panel depth of five
216 (for the five initial dog breeds in the ascertainment panel). We found the maximum likelihood of
217 the true probabilities of each entry in the site frequency spectrum given our observed values of
218 entries.

219 PRFREQ was then used to estimate demographic parameters based on two site frequency
220 spectra (corrected for ascertainment bias and uncorrected). We estimated the following
221 parameters: length of the domestication event (τ_B), the bottleneck population size (ω_B), and the
222 domesticated dog population size after the bottleneck (ω) (Figure 1a & Table 2). The model that
223 best fit the observed data was determined by comparing the likelihoods of the nested models. A
224 constant population size of 21,591 in gray wolf (N_{e_WOLF}) was assumed over time, estimated from
225 Watterson’s θ ($\theta = 4N_e\mu$) calculated from pairwise estimates between all wolves and using a
226 mutation rate (μ) of 1×10^{-8} per generation (LINDBLAD-TOH *et al.* 2005). This value was re-
227 evaluated excluding populations found later to exhibit some evidence of a past contraction event
228 (see results) but was minimally different (22,600). The initiation of the domestication of

229 domestic dogs (τ) was assumed to be 15,000 years before present (OLSEN 1985; SAVOLAINEN *et*
230 *al.* 2002).

231 **Breed Formation Model:**

232 To further model the formation of breeds (Figure 1b), we used the complete sequence
233 data from Sutter *et al* (2004) from five chromosomes in five dog breeds. However, as a result of
234 breeding programs, dog breeds are highly inbred, and an individual's chromosomes are more
235 similar than expected under random mating. Since this could potentially affect our demographic
236 inferences, we attempted to reduce the effects of inbreeding within breeds by sampling one
237 chromosome per individual per breed creating a site frequency spectrum for each of 2000
238 iterations, and averaging across iterations. Because the data were generated by sequencing and
239 sample sizes of SNPs were consistent across loci, corrections for sample size and ascertainment
240 bias were not necessary as with modeling the domestication event. Likelihoods between several
241 nested models were compared to determine which model best fit the observed data (Figure 1b &
242 Table 2).

243 **Wild Canid Model:**

244 To model the formation of wild canid populations (Figure 1c), we used chromosome 1
245 sequence data generated in this study across four gray wolf populations and one coyote
246 population. To account for potential inbreeding in wild canid populations, and to ensure that
247 unknown closely-related individuals did not bias our estimates, we chose to perform the same
248 chromosome sampling done for dog breeds. As in the breed formation models, corrections for
249 sample size and ascertainment bias were not necessary.

250 Nested models identical to those examined for breed formation were used to model the
251 wild canid populations (Figure 1c & Table 2). However, models fixing the time of contraction

252 were not tested, as we did not know *a priori* whether we should expect to see a population
253 contraction or expansion nor the timing of such an event. For those populations with evidence for
254 a population decline, we tested models varying the contraction event.

255 RESULTS

256 **Genetic Diversity:**

257 To study the effects of demographic history on LD in wild canids and domestic dogs, we
258 sequenced 11,279 base pairs spanning ~5.2Mb on dog chromosome 1 (Figure S2 & Table S2). A
259 total of 92 SNP loci (Table S2 & S3) were identified of which 54 were polymorphic across four
260 gray wolf populations, 48 were polymorphic in one coyote population, and 43 were polymorphic
261 across five breeds of dog.

262 Eighteen percent of SNPs on average were shared within and between canid species with
263 gray wolf populations exhibiting the highest sharing (27%; Table S4). Interestingly, 24 loci
264 (26%) were observed to have the derived allele fixed in domestic dogs but polymorphic in gray
265 wolves which likely reflects the bottlenecks associated with domestication or breed formation.
266 The average proportion of shared haplotypes within and between species was 74%. Wolf
267 populations had the highest average percentage of haplotype sharing (90%; Table S4). Average
268 nucleotide diversity among dog breeds was significantly different from that within dog breeds (t-
269 test p-value <0.001; Table S5) and among wolf populations (p-value <0.001). Furthermore,
270 examination of the ratio of nucleotide diversity suggests a minimal loss of diversity as a result of
271 the domestication event (0.05) whereas the average loss of diversity due to breed formation was
272 much larger (0.35).

273 Using data from five chromosomes (1, 2, 3, 34, and 37), 105 of 106 loci were
274 successfully genotyped in 18 dog breeds, and a variety of wild canid species (Table S6). In the

275 most distantly related species, approximately 93% of loci in golden jackals, and 80% of loci in
276 bat-eared fox, gray fox, and island fox were successfully amplified. Observed heterozygosity
277 values ranged from 0.24 in golden jackals to 0.31, 0.29, and 0.33 in the bat-eared, gray, and
278 island fox, respectively.

279 **Population Structure:**

280 Population structure across a variety of wild canid species and domestic dog breeds was
281 explored through PCA of the genotype data (Figures 2a-d). Fourteen principal components (PCs)
282 were found to be significant for the seven canid species analyzed (Figure 2a). Domestic dog,
283 gray wolf, coyote, golden jackal, and foxes were found to separate along principal component
284 axes 1 and 2. Red wolf was found to overlap both coyote and gray wolf but more so with the
285 latter species.

286 PCA of 13 gray wolf populations revealed 11 significant principal components (Figure
287 2b). The most distinct pattern was observed along the first axis of variation separating Old World
288 and New World wolf populations. Minimal overlap between the two groups was evident. PCA
289 was then performed separately on Old and New World populations (Figures 2c & d). Seven Old
290 World gray wolf populations were found to have seven significant principal components. The
291 first axis of variation visibly separated the majority of the populations with a distinct separation
292 of Swedish gray wolves. Six New World gray wolf populations were found to have six
293 significant principal components. Isle Royale, Minnesota, and Northern Quebec define distinct
294 clusters with Alaska, Canada, and Yellowstone forming a fourth cluster.

295 PCA of 18 domestic dog breeds exhibited 15 significant principal components (data not
296 shown). There was considerable overlap between breeds. However, Akita displayed virtually no
297 overlap with any other domestic dog breed while Pekingese exhibited slight separation along the

298 first axis of variation. Along the second axis of variation, Mastiffs and to a lesser extent,
299 Portuguese Water Dogs exhibited separation from the main cluster of breeds.

300 **Ascertainment Bias:**

301 Ascertainment bias typically produces a pattern characterized by a decrease in low
302 frequency alleles and an increase in higher frequency alleles (CLARK *et al.* 2005; ROSENBLUM
303 and NOVEMBRE 2007). A shift in allele frequencies was observed between the sequence and
304 genotype data; however, there was no discernable pattern (Figure S4). Simulations showed that
305 the degree to which the difference in allele frequency affects estimates of LD varies for each
306 breed (Figure S5). Ascertainment bias in Labrador Retriever was observed to have minimal
307 effects; however, in Golden Retrievers the effect was large. Despite the variance in estimates, the
308 rank order of breeds based on estimates of LD remained the same.

309 Site frequency spectra generated from sequence and genotype data for gray wolf and
310 coyote populations were similar to the domestic dog in showing no distinguishable patterns
311 (Figure S4). When LD estimates from sequence data were compared with LD estimates from
312 genotype data, sequence data generally gave lower estimates of LD (Figure 3). Spanish gray wolf
313 was the exception with an increase in LD measured from genotyped data to sequenced data.
314 Despite the shift in LD estimates from sequence to genotype data, the rank order of LD estimates
315 of each population remained the same.

316 Given that ascertainment bias is present within the genotype dataset, we proceeded with
317 caution by focusing on general trends (e.g., strong association between the extent of LD and
318 demographic history). Additionally, we relied on estimates from re-sequencing to make unbiased
319 estimates and comparisons of genetic diversity and extent of LD between domestic and wild
320 canids.

321 **Linkage Disequilibrium:**

322 The extent of LD estimated from genotyped data in gray wolf populations ranged from
323 <10kb in Alaskan gray wolves to >5Mb in gray wolves from Isle Royale ($r^2_{0.2}$; Table 3; see
324 Figure S6 for D' estimates). The extent of LD was consistent with the known demographic
325 history of each population. Large outbreeding populations such as Alaska, Minnesota, Canada,
326 Yellowstone, and Northern Quebec exhibited such low levels of LD that the decay curves did not
327 extend to an r^2 value of 0.2 (Figure 3 and Table 3). Therefore, we take a conservative approach
328 and consider these populations to generally have LD levels lower than 10kb. However,
329 small/bottlenecked populations such as Isle Royale, Spanish, Italian, and Swedish gray wolves
330 exhibited high levels of LD ($r^2_{0.2}>500\text{kb}$). Lastly, coyotes exhibited levels of LD below an r^2
331 value of 0.2 (Figure 3) consistent with their large population size in southern California
332 (FEDRIANI *et al.* 2001; VILÀ *et al.* 1999a).

333 Estimates of LD from genotyped data in dog breeds ranged from 20kb to >5Mb ($r^2_{0.2}$;
334 Table 3). The extent of LD was found to be significantly correlated to the log of registered
335 individuals for both Kendal's tau rank correlation (p-value = 0.02) and Mantel's test (p-value =
336 0.0001). However, three breeds had sample numbers below the minimum cutoff ($n<17$) used by
337 Sutter *et al.* (2004), introducing potentially greater bias into our measures of LD. When these
338 breeds were excluded from the analysis, the correlation statistics for the remaining 14 breeds
339 were still significant (Kendal's tau, p-value = 0.003; Mantel's test, p-value = 0.0002). Thus, the
340 level of LD within dog breeds was found to be well correlated with 2006 registration numbers
341 (Figure S7).

342 Values of LD based on sequence data were highly correlated with those based on SNP
343 genotypes (Kendal's tau, p-value = 0.02; Mantel's test, p-value = 0.0001). The extent of LD

344 between species based on sequence data demonstrated that gray wolves and coyotes have less
345 LD (<10kb to 1.7Mb) than the domestic dog (785kb to >5Mb; Table 3). The extent of LD seen in
346 the Spanish gray wolf population was much higher than any other sequenced gray wolf
347 population (1.7Mb). We explored the possibility of relatedness among the samples by
348 eliminating individuals with high levels of allele sharing based on 11 microsatellite loci
349 (VONHOLDT *et al.* 2008), and confirmed that high levels of LD are still present in a sample set of
350 reduced allele sharing ($r^2_{0.2} \geq 1.5\text{Mb}$).

351 **Domestication Modeling:**

352 Parameter estimates were scaled in terms of the estimated gray wolf effective population
353 size (i.e., $\omega = N_{e\text{DOG}}/N_{e\text{WOLF}}$) and multinomial calculations were found to be qualitatively similar
354 to Poisson calculations (see supplement Tables S7-S8 & Figures S8-S10). The Contraction at
355 Fixed Time model, with a single contraction event fixed at 15,000 years ago, explained the data
356 significantly better than the null model of constant population size (Table 4). This applied for
357 both the ascertainment bias corrected (p-value = 2.27×10^{-6}) and uncorrected data sets (p-value =
358 4.01×10^{-8}). The corrected Poisson calculations suggest that this contraction was followed by a
359 population expansion (Bottleneck of Fixed Size, Table 4), although the improvement in the
360 model fit is slight (p-value = 0.033) and unlikely to be significant after correcting for linkage in
361 the dataset. Therefore, we focus on the Contraction at Fixed Time model findings. The estimate
362 of ω for the Poisson calculation of the Contraction at Fixed Time model was 0.23 for the
363 uncorrected data, indicating the dog ancestral population size was 0.23 times the size of the wolf
364 ancestral population, and 0.25 for the corrected data. Therefore, results suggest a single minor
365 contraction event was associated with domestication of the dog.

366 **Breed Formation Modeling:**

367 Demographic parameters based on the discussed model of breed formation were
368 estimated for each of 5 breeds from the averaged sampled site frequency spectra. Across all
369 breeds and calculations, no models had a higher likelihood than the Contraction at Fixed Time
370 model (Table 5), which indicates a contraction without a subsequent increase in population size.
371 Under the Contraction at Fixed Time model, Bernese Mountain Dog and Pekingese were
372 observed to have the largest bottleneck with a current effective population size approximately
373 0.0055 and 0.0056 that of the ancestral dog effective population (assumed to be equal to the gray
374 wolf effective population size). Labrador Retriever, Golden Retriever, and Akita exhibited a
375 weaker reduction in population size with values of 0.0095, 0.011, and 0.012, respectively.

376 Although not significantly better than the Contraction at Fixed Time model, both ω and τ
377 were optimized under the Contraction at Unknown Time model allowing examination of the
378 timing of breed contractions. Under this model, Pekingese was observed to have a severe
379 reduction in population size ~65 generations ago ($\omega=0.0035$), while the Akita and Golden
380 Retriever were observed to have similar contraction times at ~92 generations ($\omega=0.0113$ &
381 0.0100). It is important to note that when estimating both ω and τ , timing estimates may not be
382 entirely realistic, as there is a tradeoff between having a recent τ and a severe population decline
383 and having a more distant τ but less severe decline. For example, the founding prediction of 755
384 generations ago for Bernese Mountain Dog, originating from a small number of individuals and
385 maintained as a small population, is likely an overestimate. Regardless, the bottleneck at breed
386 formation across breeds is orders of magnitude more severe, and more recent, than an ancient
387 domestication event and more likely to impact differences in LD among breeds (see below).

388 **Wild Canid Modeling:**

389 As with the inference of breed formation, we used Poisson calculations to determine the
390 presence and severity of a bottleneck within gray wolf and coyote populations. Only the
391 Contraction at Unknown Time model of the Spanish and Israeli gray wolf populations was found
392 to be significantly different from the null model (Table 5). The Spanish gray wolf was observed
393 to have undergone a contraction ($\omega=0.028$) about 226 generations ago (~700 years ago) and the
394 Israeli wolf population was observed to have undergone a more mild population decline
395 ($\omega=0.25$) over 10,000 generations ago (30,000 years ago). Again, these estimates may not be
396 entirely accurate as they may represent the tradeoff between ω and τ (see above). Lastly, no
397 significant evidence was found to support a change in population size in Alaskan and
398 Yellowstone gray wolf or coyote populations.

399 DISCUSSION

400 The extent of LD and its relationship to demographic history has been well documented
401 in domesticated and model organisms (ARDLIE *et al.* 2002; DUNNING *et al.* 2000; LAURIE *et al.*
402 2007; PRITCHARD and PRZEWORSKI 2001). However, little research has been done to explore the
403 extent of LD in wild populations, particularly vertebrate species. As mentioned previously, only
404 a few studies to date have measured the extent of LD in naturally occurring vertebrate
405 populations. Utilizing SNP markers developed in the domestic dog and extensive resequencing,
406 we explored the extent of LD and modeled demographic history in several populations of wild
407 canids. Additionally, we calculated the same measures in the domestic dog for comparison.

408 Five domestic dog breeds, four gray wolf, and one coyote population were sequenced for
409 11,279 bps on chromosome 1. Levels of LD in domestic dogs were consistent with previous
410 studies (LINDBLAD-TOH *et al.* 2005; SUTTER *et al.* 2004) and in general, we found that gray wolf
411 and coyote populations exhibited lower levels of LD (<10kb to 1.4Mb) than domestic dog breeds

412 (785kb to >5Mb; Table 3). Barley (CALDWELL *et al.* 2006), soybean (HYTEN *et al.* 2007), sheep
413 (MCRAE *et al.* 2002; MCRAE *et al.* 2005), and house mice (LAURIE *et al.* 2007) display a
414 consistent pattern of reduced levels of LD in wild populations compared to their domesticates.
415 This is expected since domestication likely results in a bottleneck event. However, across wild
416 populations, demographic history can still be observed to strongly influence levels of LD. For
417 example, the Spanish wolf population had LD levels higher than some domestic dog breeds
418 ($r^2_{0.2}=1.7\text{Mb}$). In the past century, gray wolves from Spain were hunted to near extinction, but
419 have steadily risen in numbers since the enactment of hunting restrictions (RAMIREZ *et al.* 2006).
420 In contrast, Labrador Retrievers exhibited levels of LD similar to wild gray wolf populations
421 ($r^2_{0.2}=785\text{kb}$) as they are the most popular breed in the U.S. today with about 150,000 new
422 registrations per year (www.AKC.org). Lastly, coyotes were found to display the lowest levels of
423 LD ($r^2_{0.2}<10\text{kb}$) relative to all domestic dog breeds and gray wolf populations. Consistent with
424 low levels of LD, coyote population sizes are reportedly an order of magnitude greater than the
425 gray wolf (VILÀ *et al.* 1999a).

426 As seen with the sequence data, LD levels from SNP genotype data were found to
427 correspond with known demographic history for the 11 gray wolf populations. For example, the
428 Isle Royale gray wolf population is a small population of wolves that inhabit an island in Lake
429 Superior off the coast of Minnesota. The population was founded by a single breeding pair in
430 1950 (PETERSON *et al.* 1998). Previous genetic research found population heterozygosity levels
431 half that observed in the mainland progenitor (Wayne *et al.* 1991). The extent of LD in the Isle
432 Royale population ($r^2_{0.2}>5\text{Mb}$) is consistent with that expected in small and/or severely
433 bottlenecked populations (GAUT and LONG 2003; MUELLER 2004; PRITCHARD and PRZEWORSKI
434 2001). Other populations that are known to have undergone a contraction or have a history of

435 small population size had high levels of LD (Spanish, Swedish, and Italian gray wolves; (for
436 supporting demographic and genetic research see: FABBRI *et al.* 2007; LEHMAN *et al.* 1992;
437 RAMIREZ *et al.* 2006; VILÀ *et al.* 1999a; WAYNE *et al.* 1992). At the other end of the spectrum,
438 populations of Alaskan, Canadian, and Northern Quebec gray wolves have been large and of
439 constant size for a long time, and exhibit low levels of LD (MUSIANI *et al.* 2007; WECKWORTH *et*
440 *al.* 2005). Supporting this finding, genetic studies (ROY *et al.* 1994; VILÀ *et al.* 1999b; WAYNE *et*
441 *al.* 1992) of Alaskan and Northern Canadian gray wolf populations found high variability and
442 reduced population differentiation suggesting a large population size and higher levels of gene
443 flow than among European wolf populations which were more structured. Similarly, LD
444 estimates in dog breeds from SNP genotype data corroborate findings from sequence data as
445 exemplified by a significant correlation to popularity of the breed based on registration numbers
446 (Figure S7). Thus, the extent of LD measured from the SNP genotype data also support the
447 correlation between LD and demographic history in wild and domestic populations.

448 **Demographic modeling:**

449 Previous studies based on mtDNA analysis (SAVOLAINEN *et al.* 2002; VILÀ *et al.* 1997)
450 have indicated that four to six matrilineal gray wolf were involved in the founding of the
451 domestic dog. In contrast, analysis of major histocompatibility (MHC) loci suggested several
452 hundred founders or extensive backcrossing with wild canids is needed to explain present day
453 diversity in domestic dogs (VILÀ *et al.* 2005). Linbladh-Toh *et al.* (2005) found evidence for two
454 major bottlenecks in modern dog breeds, the first occurring as a result of domestication from
455 wolves, supported by short range LD estimates, and the second occurring as a result of breed
456 formation, supported by long range LD. Linbladh-Toh *et al.* (2005) simulated the demographic
457 history of domestic dogs over a coarse grid of demographic parameter values, and compared the

458 observed and simulated rates of pairwise polymorphism across ten 15 Mb regions. They then
459 selected the domestication parameters for which the simulations resulted in polymorphism values
460 that were the closest to observed values. Although they did find evidence for two major
461 bottlenecks, they did not use a rigorous likelihood framework, and thus are not able to perform
462 any hypothesis testing or formal model selection. For our analysis, we searched a denser grid of
463 domestication parameter values and examined the site frequency spectrum of dogs rather than
464 pairwise polymorphism. For domestication events with parameters that were searched over this
465 grid, we calculated the likelihood of the observed domesticated dog site frequency spectrum. In
466 this likelihood framework, we were able to perform nested likelihood ratio tests to test the null
467 hypothesis of constant population size and make meaningful comparisons between models.

468 From our demographic modeling, we found evidence for a modest population contraction
469 approximately 15,000 years ago (5,000 generations ago) and severe contractions at breed
470 formation. The contractions due to breed formation were found to be an order of magnitude
471 greater than the domestication contraction based on analysis of the site frequency spectra. From
472 nucleotide diversity estimates, only a 5% reduction in diversity was observed as a result of
473 domestication whereas an average of 35% of nucleotide diversity was lost due to breed
474 formation. This severe contraction at breed formation was expected, as continued inbreeding
475 within a given breed may act to maintain a small effective population size even if the census
476 population size has actually increased since breed formation. The absence of a strong signal for a
477 contraction at domestication may reflect continued interbreeding between early dogs and wolves
478 or multiple domestication events (RANDI and LUCCHINI 2002; TSUDA *et al.* 1997; VILÀ *et al.*
479 1997). Indeed, high levels of diversity observed in domestic dogs may have been maintained

480 through a modest population bottleneck, backcrossing with wolf populations, and rapid
481 population expansion (VILÀ *et al.* 2005; WAYNE and OSTRANDER 2007).

482 Lastly, demographic modeling of the site frequency spectra of wild canid populations and
483 dog breeds were found to be concordant with estimates of LD and known population history. In
484 wild canid populations, a significant population decline was observed for the Spanish gray wolf
485 and to a lesser extent the Israeli gray wolf population, which was expected from known historical
486 data. Furthermore, neither coyote nor Alaskan and Yellowstone gray wolf populations showed
487 significant evidence of a population size change. In modeling the demographic history of
488 domestic dog breeds, Pekingese and Bernese Mountain Dog exhibited the greatest population
489 contraction and more modest contractions were observed in Golden Retriever and Labrador
490 Retriever. The strong concordance observed in this study between the extent of LD, demographic
491 modeling, and known demographic history supports the use of LD to infer population history not
492 only in model organisms but also in wild populations.

493 **Population Structure:**

494 Eighty percent or greater of SNPs that were discovered in dogs successfully amplified in
495 the most distantly related species and polymorphism levels ranged from 25% to 40%. Genetic
496 isolation and/or admixture revealed in the PCA was consistent with previous studies (LEHMAN *et*
497 *al.* 1992; LEONARD *et al.* 2005; ROY *et al.* 1994; VILÀ *et al.* 1999a; WAYNE *et al.* 1992; WAYNE
498 *et al.* 1991). Within gray wolves, PCA identified strong geographic differentiation between Old
499 and New World populations as well as populations within each continent. Similar relationships
500 have been observed in mtDNA studies of gray wolves (ROY *et al.* 1994; VILÀ *et al.* 1999a;
501 WAYNE *et al.* 1992). Patterns in the PCA plots were consistent with previous phylogenetic
502 studies (LEONARD *et al.* 2005; LINDBLAD-TOH *et al.* 2005; ROY *et al.* 1996; VILÀ *et al.* 1999a).

503 For example, PC one supports the fundamental genetic distance between wild canids and
504 domestic dogs. PC two distinguishes wild canids from each other with coyotes and golden
505 jackals positioned nearest to the gray wolves and red wolves overlapping coyotes and gray
506 wolves. The overlap of red wolves with both species is consistent with extensive hybridization in
507 the past (WAYNE and JENKS 1991). The high degree of SNP amplification success between
508 species suggests that dog-derived SNP markers may be useful in mapping phenotypic traits in
509 wild canid species. To support this conclusion, Kukekova et al. (2007) used dog derived
510 microsatellite markers to develop a genetic map for the silver fox and Sacks and Louie (2008)
511 and Seddon et al.(2005) sequenced SNP loci from the dog genome to develop new SNPs for
512 genetic studies in gray wolf, coyote, red fox, and gray fox.

513 **Conclusions:**

514 The extent of LD in natural vertebrate populations has been difficult to assess in the past
515 because large scale genomic surveys were only possible in model species. However, with the
516 availability of high throughput genotyping and information from genome sequencing projects, a
517 new era has emerged in the genetic characterization of natural populations. Utilizing these
518 resources, we have estimated LD in 11 natural populations of gray wolf, one population of
519 coyote and 18 dog breeds. Additionally, because a causal relationship exists between LD and
520 population history, we have made inferences about the demographic and evolutionary processes
521 in wild and domestic canids. Our results suggest that a relatively minor population contraction
522 was associated with domestication in dogs and that genetic variation was preserved in the rapid
523 expansion that followed. However, this variation is now partitioned in dog breeds which
524 generally have high and variable amounts of LD. The high level of LD in some wolf populations
525 further suggests the possibility of trait mapping in natural populations. For example, in North

526 America, approximately half of wolves are dark colored (ANDERSON *et al.* in press; MUSIANI *et*
527 *al.* 2007), and given the recent identification of coat color mutants in dogs associated with black
528 color (CALDWELL *et al.* 2006), similar mutants may now be identified through association studies
529 in wild wolves. Finally, we demonstrate how simulation models in general can be used to make
530 inferences about population demography and show that predictions generally fit with observed
531 levels of LD and known population history. Consequently, our approach may have wide
532 applicability to other species with extensive genomic resources and their close relatives.
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Table 1 Samples genotyped and sequenced.

Genotyping (106 SNP Loci)			Sequencing (18 Amplicons)**		
# of Populations	# Individuals	Species	# of Populations	# Individuals	Species
14	344	Gray Wolf	4	73	Gray Wolf
1	18	Coyote	1	17	Coyote
18	546	Domestic Dog Breeds	5	97	Domestic Dog Breeds*
1	18	Golden Jackal	1	1	Golden Jackal
1	20	Bat-eared Fox			
1	29	Gray Fox			
1	26	Channel Island Fox			
	1001			188	

** Subset of samples that were SNP genotyped.

* Same samples set used in Sutter et al. (2004).

Table 2. Parameters and demographic models tested.

	Parameters				Demographic Models		
					Domestic Dog		Wild Canids
					Breed		Wild Population
Nested Likelihood Models	τ	ω	τ_B	ω_B	Domestication	Formation	History
Constant Population Size	-	-	-	-	X	X	X
Contraction at Fixed Time	fixed	vary	-	-	X	X	-
Contraction at Unknown time	vary	vary	-	-	X	X	X
Bottleneck of Fixed Size	fixed	vary	vary	fixed	X	-	-
Bottleneck of Unknown Size	fixed	vary	vary	vary	X	-	-
Bottleneck of Fixed Length	vary	vary	fixed	vary	-	X	-
Bottleneck of Unknown Length	vary	vary	vary	vary	-	X	X

- not applicable or not tested.

τ , time of contraction event in number of generations.

τ_B , length of time the contraction occurred in number of generations.

ω , effective population size following the contraction scaled in terms of the ancestral wolf/dog effective population size.

ω_B , effective population size during the contraction scaled in terms of the ancestral wolf/dog effective population size.

Table 3. Estimated extent of linkage disequilibrium measured as the distance at which r^2 decays to 0.2.

Breed	Domestic Dog			Wild Canid		
	Genotype Data	Sequence Data	2006 AKC Registration	Populations	Genotype Data	Sequence Data
Labrador Retriever	20kb	785kb	123,760	Alaska	<10kb	<10kb
Saint Bernard	83kb		3,828	Canada	<10kb	
Pomeranian	200kb		18,218	Yellowstone	<10kb	<10kb
Poodle	250kb		29,939	Minnesota	<10kb	
Standard Poodle	318kb			Israel (older)	<10kb	
				Northern		
Toy Poodle	415kb			Quebec	<10kb	
Dachshund	427kb		36,033	Israel (2006)	564kb	241kb
Whippet	500kb		1,836	Spain	906kb	1.7Mb
Pembroke Welsh						
Corgi	639kb		10,250	Sweden	1Mb	
Old English	672kb		1,403	Italy	3.2Mb	

Sheepdog					
Miniature Poodle	672kb			Isle Royale	*
Scottish Terrier	716kb		3,545		
Doberman Pinscher	826kb		11,546	Coyote	<10kb <10kb
Portuguese Water Dog	846kb		1,454		
Golden Retriever		1.4Mb	42,962		
Akita	1.3Mb	1.4kb	2,457		
Border Terrier	1.3Mb		873		
Briard	2Mb		284		
Irish Wolfhound	2.2Mb		1,016		
Basset Hound	2.4Mb		7,844		
Pekingese	4.2Mb	*	3,155		
Mastiff	*		6,913		
Bernese Mountain Dog		*	3,714		

* indicates the decay curve did not reach an r^2 of 0.2.

Models	Contraction at Fixed Time			Contraction at Unknown time			Bottleneck of Fixed Size					
	df = 1			df = 2			df = 2					
	$\tau = \text{fixed}$	$\omega = \text{vary}$	p-value	$\tau = \text{vary}$	$\omega = \text{vary}$	p-value	$\tau = \text{fixed}$	$\omega = \text{vary}$	$\tau_B = \text{vary}$	$\omega_B = 0.1$	p-value	$\tau = \text{fi}$
Uncorrected	5000	0.23	4.01×10^{-8}	2245	0.12	0.51	5000	64.5	1835	0.1	0.51	500
Corrected	5000	0.25	2.27×10^{-5}	16193	0.38	0.11	5000	2.5	2591	0.1	0.03	500

See table 2 for description of parameters.

p-values calculated from likelihood ratio test, where a p-value < 0.05 indicates the alternative model fits the data better than the previous model.

null model ($\tau = \infty$ and $\omega = 1$).

Table 5. Poisson calculated parameter estimates under nested likelihood models of breed formation and wild canid population history.

Breed / Wild Canid Population	Contraction at Fixed Time			Contraction at Unknown Time		
	df = 1			df = 2		
	$\tau = \text{fixed}$	$\omega = \text{vary}$	p-value	$\tau = \text{vary}$	$\omega = \text{vary}$	p-value
Akita	100	0.012	6.74×10^{-8}	92	0.011	0.96
Bernese Mountain Dog	100	0.006	0	756	0.039	0.77
Golden Retriever	100	0.011	4.72×10^{-10}	92	0.01	0.99
Labrador Retriever	100	0.01	1.03×10^{-10}	367	0.035	0.83
Pekingese	100	0.006	0	65	0.004	0.87
Alaska Wolf	-	-	-	97	0.053	0.61
Israel Wolf	-	-	-	10796	0.25	0.002
Spain Wolf	-	-	-	227	0.028	0.014
Yellowstone Wolf	-	-	-	16	0.045	0.99
Coyote	-	-	-	20512	1.63	0.26

See table 2 for description of parameters.

p-values calculated from likelihood ratio test, where a p-value < 0.05 indicates the alternative model fits the data better than the previous model.

null model ($\tau = \infty$ and $\omega = 1$).

Figure 1. Demographic model of (a) dog domestication, (b) dog breed formation, and (c) wolf population size change. N_{eWOLF} , N_{eB} , N_{eDOG} , N_{eBreed} , N_{eWOLFP} , and N_{eWOLFA} are the effective population sizes of the gray wolf (constant over time), of the population during the bottleneck, the effective population size of dogs, the effective population size of breeds, the effective population size of gray wolves prior to a population size change, and the effective population size of wolves after a population size change, respectively. τ is the time of the domestication event from the present, and τ_B is the bottleneck duration.

Figure 2. Principal component analysis of SNP genotype data within and across species: (a) all species; (b) Gray wolves; (c) Old World Wolves; (d) New World Wolves.

Figure 3. Decay plots of LD (r^2) estimates from sequence data, and genotype data in: (a,b,c) Alaskan gray wolf; (d,e,f) Israeli gray wolf; (g,h,i) Spanish gray wolf; (j,k,l) Yellowstone gray wolf; and (m,n,o) Coyote. The 1st column of data represents decay plots from chromosome 1 sequence data. The 2nd column of data represent data from SNP genotypes on all 5 chromosome, and the 3rd column of data represents decay plots from SNP genotypes just on chromosome 1. Gray diamonds are pairwise r^2 . Black squares are average r^2 for each distance group for which a logarithmic trend line was fitted to the data (black line).





