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Strong artificial selection in domestic mammals did not result in an increased recombination rate

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Abstract

Recombination rates vary in intensity and location at the species, individual, sex and chromosome levels. Despite the fundamental biological importance of this process, the selective forces that operate to shape recombination rate and patterns are unclear. Domestication offers a unique opportunity to study the interplay between recombination and selection, particularly due to Hill-Robertson interference, which should be important when many linked loci are repeatedly the target of selection. In domesticates, intense selection for particular traits is imposed on small populations over many generations, resulting in organisms that differ, sometimes dramatically, in morphology and physiology from their wild ancestor. Although earlier studies suggested increased recombination rate in domesticates, a formal comparison of recombination rates between domestic mammals and their wild congeners was missing. In order to determine broad-scale recombination rate, we used immunolabeling detection of MLH1 foci as crossover markers in spermatocytes in three pairs of closely related wild and domestic species (dog and wolf, goat and ibex, sheep and mouflon). In the three pairs, and contrary to previous suggestions, our data show that contemporary recombination rate is higher in the wild species. Subsequently, we inferred recombination breakpoints in sequence data for 16 genomic regions in dogs and wolves, each containing a locus associated with a dog phenotype potentially under selection during domestication. No difference in the number and distribution of recombination breakpoints was found between dogs and wolves. We conclude that our data indicate that strong directional selection did not result in changes in recombination in domestic mammals, and that both upper and lower bounds for crossover rates may be tightly regulated.

Introduction

In the last few years, significant progress has been made in the understanding of recombination. This process of fundamental biological and evolutionary importance contributes to the proper disjunction of homologous chromosomes during the first meiotic division in many eukaryotes, and influences genomic architecture through allele shuffling and genome rearrangements. At the molecular level, many of the proteins involved have been identified and analyzed (Page and Hawley 2003; Tease and Hultén 2004; Baudat et al. 2013), and considerable variation has been found in recombination location and rate within and across individuals. However, the selective forces that might be important in shaping recombination rate and patterns are still unclear. While the physiological and mechanistic constraints that operate at the molecular level to ensure the proper disjunction of chromosomes condition recombination, observations related to the intraspecific and interspecific heterogeneity in recombination rate and patterns are key to understand the selective pressures that may affect recombination over different genomic scales. Selection may operate to ensure the proper disjunction of chromosomes and thus reduce the rate of aneuploidy, to maintain genome integrity by lowering recombination rate locally where harmful effects such as changes in gene dosage and missense mutations may be the outcome, and by acting on recombination modifiers that may increase or decrease recombination rate and thus the degree of association between loci (Coop and Przeworski 2007).

Heterogeneity in recombination rate and patterns is observed at many levels. Recombination preferentially occurs, at least in certain organisms, in localized regions of the genome termed recombination hotspots (Arnheim et al. 2003), which seem to be ubiquitous in mammals (Kauppi et al. 2004). In humans, recombination mostly occurs in regions 1-2 kb long, 60-200 kb apart, where recombination rates can be 10-1,000 times higher than in surrounding areas (Kauppi et al. 2004; Coop and Przeworski 2007). Hotspot location is associated with a consensus sequence in humans (Myers et al. 2008) and with a different one in yeast (Steiner and Smith 2005), and recombination rate is correlated with nucleotide diversity and GC-content and

increases from the centromere to the telomere in many organisms, including, for example, yeast, rodents and humans (Kauppi et al. 2004; Coop and Przeworski 2007). There are also differences in recombination rate and location associated to the sexes (Lenormand and Dutheil 2005; Coop and Przeworski 2007); for example, in fish and most eutherian mammals studied, females have longer genetic maps, while in other instances the opposite is true or there are no differences between the sexes (Hansson et al. 2005; Calderón and Pigozzi 2006; Poissant et al. 2010; Samollow 2010; van Oers et al. 2014). Across species, recombination rates examined in 5 Mb orthologous regions of mouse, rat and human were found to be weakly correlated (Jensen-Seaman et al. 2004), and humans and chimpanzees do not share hotspot locations, suggesting that their location evolved over time scales that are shorter than the separation of these two species (Winckler et al. 2005; Auton et al. 2012), about 5-6 million years ago (Patterson et al. 2006), despite 99% identity at the sequence level. Several studies indicated that recombination rate is heritable (Charlesworth and Charlesworth 1985; Kong et al. 2002; Dumont et al. 2009; Tortereau et al. 2012) and recent research has shown that variation in the zinc-finger domain of a protein called PRDM9 plays a role in the localization of recombination hotspots in humans and mice (Baudat et al. 2010; Berg et al. 2010; Berg et al. 2011; Brick et al. 2012) .

An extensive body of theoretical work has been dedicated to identify the conditions under which changes in recombination rate may be beneficial, and thus spread, in a population. The key idea is that recombination breaks up the association between loci, thus contributing to genetic diversity through the creation of new combinations of alleles that may result in novel phenotypes, or in new epistatic interactions, which will affect the organism's fitness and ability to respond to selection. Most successful and realistic explanatory models revolve around the idea of the presence of modifier loci that alter the frequency of recombination (Otto and Lenormand 2002). For example, a genetic modifier that increases recombination, even if it would decrease mean fitness in the short term, may be advantageous if it increases the variance in fitness, which would lead to an increased ability to respond to selection (Otto and Lenormand 2002; Butlin 2005). Higher recombination rate would be advantageous in small populations

subject to strong selection due to Hill-Robertson interference, in a situation of weak negative epistasis between loci, or in spatially heterogeneous habitats when alleles are selected in the same direction in each population (either beneficial or deleterious in all populations), but with effects that co-vary negatively across populations (e.g. for loci A and B, selection is stronger in habitat 1 for locus A and in habitat 2 for locus B) (reviewed in Otto and Barton 2001; Otto and Lenormand 2002; Ross-Ibarra 2004; Butlin 2005; Coop and Przeworski 2007; T. Lenormand, pers. com.).

Domesticates offer a unique opportunity to study the interplay between recombination and selection. Domestication can be viewed as a long term experiment in which animals and plants are subjected to intense selection for particular traits, in small populations and during thousands of generations, resulting in individuals that may differ, sometimes dramatically, in morphology and physiology from those in other populations and from their wild ancestors. It has been hypothesized that recombination played a key role in this process (reviewed in e.g. Ross-Ibarra 2004; Butlin 2005). According to Ross-Ibarra (2004), Rees and Dale (1974) proposed an increase in recombination rate in domestic species as a result of the selective forces imposed, while Gornall (1983) also expected a higher recombination frequency in domestic species, but hypothesized that high recombination rate would predate domestication, as higher recombination rate would increase response to selection and thus contribute to the success in domestication. Recombination would be particularly beneficial when genetic variability is limited by linkage disequilibrium subsequent to extensive population bottlenecks and drift (reviewed in Ross-Ibarra 2004). In small populations subject to strong selection, such as is the case for domestic species, simulations showed that high recombination would be beneficial (Otto and Barton 2001) and, in laboratory experiments, recombination increased in small animal populations subjected to strong selection for an unrelated trait (reviewed in Otto and Barton 2001; Otto and Lenormand 2002; Bell 2008). Increased recombination would be advantageous in breaking up the random association of alleles generated by drift, thus reducing Hill-Robertson interference (Otto and Lenormand 2002). However, a different hypothesis proposed a

reduced recombination in domesticates in order to protect from maladaptive gene flow from wild relatives, such as between loci that were positively selected in domestic species (Lenormand and Otto 2000).

The wealth of theoretical studies dealing with the conditions for the evolution of recombination rates in small populations and domesticates contrasts with the few empirical studies. Ross-Ibarra (2004) used the number of chiasmata as a proxy for recombination rate and compared chiasma frequencies for 196 plants (including domesticated species and their wild progenitors and congeners); no support was found for the preadaptation hypothesis (comparing wild progenitors and congeners), and only a modest increase in recombination rate in domesticates as compared to wild congeners. The study concluded that "recombination rate is likely of little importance" in relation to plant domestication (Ross-Ibarra 2004). The study of Burt and Bell (1987), in which chiasmata counts for domestic mammals are reported, is often cited as evidence that domestic animals have higher recombination rate than their wild counterparts (see Schmidt-Hempel and Jokela 2002; Dumont and Payseur 2008; Groenen et al. 2009; Backström et al. 2010; Poissant et al. 2010; Smukowski and Noor 2011). However, domestic species were not compared with wild relatives in this study. A recent study has demonstrated a strong phylogenetic effect in recombination rate (Dumont and Payseur 2008), and a comparison of changes in the rates of recombination should take this effect into account. Additionally, in a study comparing linkage maps, the assumption of higher recombination rate in domesticates was found not to be supported in insects (Wilfert et al. 2007). Therefore, the evidence for an increase in recombination rate in domestic animals is inconclusive.

Here we measured recombination rate in domestic mammals and their wild counterparts using cytogenetic approaches. We counted the number of MLH1 foci, a marker of crossover sites (e.g., Lynn et al. 2004), along the synaptonemal complex in spermatocyte spreads of domestic mammals and wild relatives: dogs (*Canis familiaris*) and gray wolves (*C. lupus*), goats (*Capra hircus*) and ibexes (*C. pyrenaica*), and sheep (*Ovis aries*) and mouflons (*O. musimon*). MLH1 is

a mismatch repair protein that is recruited to crossover sites during the pachytene stage of prophase I.

Counting MLH1 foci provides an estimate of broad-scale contemporary recombination rate, but it is not informative of recombination rate throughout the population history or the fine-scale location of recombination breakpoints. Statistical advances in coalescent modeling of genome-wide polymorphism data allow the estimation of fine-scale recombination rates averaged over many generations (Stumpf and McVean 2003; McVean et al. 2004; Auton and McVean 2007). Artificial selection may have favored individuals with increased recombination around genes associated with distinct phenotypes, so that these genes would be decoupled from surrounding regions, making artificial selection more efficient.

The association of recombination with genes associated with distinct phenotypes can be investigated in dogs. The dog and the gray wolf represent an interesting and valuable system to study the evolution of recombination at the fine scale. The dog has been subjected to intense artificial selection and it is the most phenotypically diverse mammal. The morphological (Wayne 1986a, b), physiological and behavioral (Coppinger and Coppinger 2001) variation present among dogs is greater than across the entire family Canidae, which includes 36 species such as raccoon dog, foxes, wolves, jackals and coyotes, which have evolved over about 15 million years. A wealth of genetic resources exist for the dog and, in terms of recombination, Burt and Bell (1987) reported that the dog had the highest number of chiasmata among all the mammals included in their study. Moreover, canids are the only known eutherian mammals to carry a *Prdm9* which has acquired disruptive mutations (Muñoz-Fuentes et al. 2011; Ponting 2011; Axelsson et al. 2012) (a marsupial, the opossum *Monodelphis domestica*, has a *Prdm9* which lacks zinc fingers; Ponting 2011). In order to investigate this hypothesis, that is, whether artificial selection favored individuals with increased recombination around genes associated with distinct phenotypes, we selected 16 genomic regions associated with phenotypic characters that are candidates to have been selected during dog domestication (e.g., body size, coat type,

color; Table 1), potentially also by early breeders, as archaeological remains suggest for skeletal and size differences (Clutton-Brock 1999). We then used sequence data to test the hypothesis that increased recombination had been favored in these regions in dogs as compared to wolves. Therefore, in addition to the cytological techniques mentioned above to study contemporary patterns of broad-scale recombination rate in three domestic mammals and their wild congeners, we also investigated patterns of fine-scale recombination and the distribution of recombination breakpoints in both dogs and wolves around loci underlying potentially selected phenotypes in dogs.

Results

Cytological estimates of contemporary recombination rate

We estimated the number of genome-wide crossover events by counting the number of MLH1 foci along synaptonemal complexes in spermatocytes. We collected testes, and obtained good quality cell preparations for 6 dogs, 2 wolves, 6 goats, 6 ibexes, 6 sheep and 5 mouflons (Table S1). We used fluorescently labeled antibodies to mark MLH1 and chromosome axes (Table 2, Fig. 1). Experiments were also carried out on pig (*Sus domesticus*) and wild boar (*S. scrofa*) samples, but MLH1 labeling failed, which suggests that the antibodies (three different ones were tested) did not recognize pig and wild boar MLH1 proteins. Although MLH1 is well conserved across mammals, some key differences exist, and it is possible that the antibodies we used to detect MLH1 recognize an immunogenic peptide (or several) that is absent or is different in pig and boar.

Dog spermatocytes contained on average 38.89 ± 0.87 MLH1 foci per cell (mean ± SD calculated over the individual means, Table 2) (Fig. 1A-C), while wolf spermatocytes contained on average 40.94 ± 1.61 MLH1 foci per cell (Fig. 1B-C). We used generalized linear

mixed models to account for mixed effects (species as a fixed factor and individual as a random factor) on the number of MLH1 foci, and found that species was a significant factor explaining the variation in the data ($P = 0.024$). Similarly, analyses of goats and ibexes showed that the average number of foci per cell was higher in the wild species (61.24 ± 4.03 for goat, 64.74 ± 1.08 for ibex; Fig. 1D-F), and again species was significant at explaining the variation ($P = 0.037$). Likewise, sheep and mouflons yielded an average number of MLH1 foci higher in the wild species (63.47 ± 3.42 for sheep, 69.03 ± 2.49 for mouflon; Fig. 1G-I), and again species significantly explained the variation present in the data ($P = 0.002$). In both ungulate pairs, the inter-individual variation was larger across the domestic species (Fig. S1B,C). Thus, contrary to previous proposals based on the study of chiasma numbers, our results indicate that wild species had higher numbers of crossover markers than their domestic counterparts.

Given that recombination correlates with the number of chromosomes and it is proportional to the number of chromosome arms, at least in mammals (Pardo-Manuel de Villena and Sapienza 2001), we calculated the mean number of MLH1 foci (inferred crossovers) expected per chromosome arm. The haploid number of chromosomes for dog and wolf is 39, and all autosomes are acrocentric, thus we calculated an average of 1.00 and 1.05 crossovers per chromosome arm in dogs and wolves, respectively (Table 2). The haploid number of chromosomes for the two *Capra* and *Ovis* species is 30 and 27, respectively (all metacentric), thus we inferred an average of 1.02 and 1.08 crossovers per arm in goat and ibex, and 1.18 and 1.28 in sheep and mouflon, respectively.

Population-genetic estimates of historical breakpoints and recombination rate

We investigated whether artificial selection would have favored increased recombination in dogs, as compared to wolves, around loci associated with traits potentially subjected to intense selection in dogs. We sequenced 16 genomic regions, each containing a locus associated to a distinct dog phenotype (totaling ca. 200-300 Kbp each) (Table 1), and we inferred

recombination breakpoints from sequence data in both dogs and wolves. The number of segregating sites per region ranged between 46 (chr10) and 1445 (chr27) (Table S2), and was similar for a given orthologous region across species or populations (wolf samples were grouped into populations with sample size similar to the sample size in dogs). In general, the number of segregating sites was lowest in dogs, and was followed in increasing order by the wolves from Spain + Italy, Sweden + Finland, and North America. We identified haplotype blocks using LDheatmaps for data previously adjusted for the same number of markers (SNPs). These maps showed more linkage disequilibrium in dogs than in wolves and, in general, a linkage block observed in wolves could also be observed in dogs, but not the reverse (Fig. S2). We estimated the number of historical recombination events using RDP3. These ranged from 2 (chr10) to 87 (chr27) (Table S2), and significantly correlated with the number of segregating sites present in that fragment ($P < 0.0001$) (Fig. 2). This is expected, as a recombination event between two identical sequences is undetectable. An analysis of covariance indicated non-significant differences between the slopes (Fig. 2, $P = 0.4$), suggesting that there were no differences in the rate of recombination in these regions across dogs and wolves.

We used LDhat to obtain estimates of mean population recombination rate, ρ, between adjacent SNP pairs for each of the 16 targeted genomic loci (Fig. 3, Fig. S3). We counted the number of **h**igher-**t**han-**a**verage **r**ecombination rate peaks (HTAR peaks) in two flanking windows of 70 kb each and a central window containing the locus potentially under selection in dogs, wolves from Europe and wolves from North America. We then compared the ratio between HTAR peaks around the locus (central window) and those in the remaining sequence (flanking windows) for dogs and each group of wolves. Our results showed no significant differences for the distribution of peaks in these regions between dogs and each group of wolves for any of the 16 genomic regions studied (Fisher's exact probability test, $P > 0.05$ for 48 comparisons). When the 16 genomic regions were considered together, the differences were again non-significant (for dogs and North American wolves, $P = 0.822$; for dogs and European wolves, $P = 0.831$;

and for dogs and all wolves, $P = 1.000$). Although this lack of differences could be partly due to limited detection power due to the small number of genomes and/or recombination breakpoints, we noted that the proportion of regions with high recombination in the central portion of the sequence was higher in dogs than in wolves for close to half of the comparisons (higher for dogs in 10 out of 16 comparisons when comparing with American wolves, 9 out of 16 when comparing with European wolves or all wolves), as would be expected if the recombination events were randomly distributed. Therefore, our results failed to support the notion of an increased recombination rate in dogs relative to wolves in regions potentially associated with selected phenotypes.

Discussion

Reduced recombination rate in domestic mammals as compared to their wild relatives

Analysis of the number of MLH1 foci as markers for crossover events showed that, for the three domestic-wild species pairs examined here, dog vs. wolf, goat vs. ibex, and sheep vs. mouflon, the wild species had higher number of crossovers per cell than the domestic counterpart. Our data accounted for an average of crossovers per bivalent that ranged between 1 and 2.56 (the autosomes of dogs and wolves are acrocentric, see Table 2), and between 1.00 and 1.28 per chromosome arm, in agreement with the requirement of one crossover per arm (except short arms in acrocentric chromosomes) for the correct segregation of chromosomes during meiosis (Hultén 1974; Pardo-Manuel de Villena and Sapienza 2001).

The number of MLH1 foci as an estimate of broad-scale recombination rate

Our estimates for broad-scale recombination rate based on the number of MLH1 foci for the dog are similar to those previously reported in cytological studies in this species (Wada and Imai

1995; Basheva et al. 2008) and, for goat and sheep they are similar or slightly higher than the mean chiasma counts for both spermatocytes and oocytes previously obtained from diplotene, diakinesis and metaphase I stage cells (Datta 1970; Jagiello et al. 1974; Logue 1977; Long 1978). These data are in agreement with the chiasma counts reported in Burt and Bell (1987), which were mostly based on the same studies for goat and sheep (A. Burt, pers. comm.). However, the dog estimate reported by Burt and Bell (1987) differs by 2-fold from estimates in this study and the other studies mentioned above, and was based on chiasma observations carried out on three male dogs corresponding to three breeds (Ahmed 1941; A. Burt, pers. comm.). It is well possible that a technical problem resulted in an overestimation in that study.

Concern has been expressed as to whether chiasma numbers are good indicators of genetic length (e.g., Hultén 1974; Wada and Imai 1995), and mapping crossovers using MLH1 foci is now established as a more accurate procedure than using chiasma counts. True chiasmata can be identified with confidence only after the complete dissolution of the synaptonemal complex and the condensation of chromosomes upon entry into meiotic metaphase. Unfortunately, the condensed nature of the chromosomes at this stage makes it difficult to accurately identify chiasmata from cell preparations alone in the absence of molecular markers. In addition, the presence of pseudochiasmata due to residual synapsis between homologous chromosome axes and the twisting of the bivalents in diplotene stage cells during prophase I of meiosis may lead to an overestimation of chiasma numbers (Hultén 1974; Wada and Imai 1995). Indeed, the numbers of perceived "chiasmata" decrease through diplotene, diakinesis and metaphase I (Datta 1970), although the numbers of crossovers are expected to remain the same. In mammals, most crossovers are formed through an MLH1-dependent pathway, while a marginal fraction depends on MUS81 activity (Holloway et al. 2008). There is now good evidence that MLH1 foci recognize the sites of meiotic exchange and provide an estimate of recombination rate that avoids the ambiguities associated with chiasma counts (Baker et al. 1996; Barlow and Hultén 1998; Anderson et al. 1999; Lynn et al. 2004; Cohen and Holloway 2010). Therefore, to count

the number of MLH1 foci on chromosome axes in meiocytes, as we have done in this study, is a more precise way of estimating broad-scale recombination rate than chiasma counts.

Linkage maps for dog, goat and sheep and MLH1 counts provide similar estimates of the number of crossovers

Availability of male linkage maps for dog, goat and sheep allowed us to compare estimates of crossovers based on map length with the average number of crossovers estimated from the number of MLH1 foci. The number of crossovers based on the count of MLH1 foci we obtained for dogs suggests that each dog chromosome pair usually contains just one crossover, which agrees with estimates based on the male dog linkage map, in which 1910 cM (Wong et al. 2010) would account for 0.98 crossover per chromosome pair (about one crossover for 50 cM over 39 chromosome pairs). For the two domestic *Capra* and *Ovis* species, 2.0 and 2.4 crossovers per chromosome pair were estimated from the number of MLH1 foci, respectively, which are similar to the number of crossovers estimated from the male linkage map (goat, 2737 cM, Schibler et al. 1998; sheep, 3876 cM, Maddox and Cockett 2007) of 1.8 and 2.9 crossovers per chromosome pair for goat and sheep (30 and 27 chromosome pairs). Therefore, the average numbers of crossovers per chromosome pair as inferred from spermatocyte MLH1 counts in this study were in agreement with those estimated from male linkage maps.

In this study we have only attempted to estimate recombination rate in males. It could be claimed that the recombination rate could still be larger in domestic mammals considering only recombination in females. However, linkage maps for the dog and the sheep have also been obtained for females (2388cM, Wong et al. 2001; 3278 cM, Maddox and Cockett 2007), which account for 1.2 crossovers per chromosome arm. Considering the agreement between MLH1 estimates and recombination rate estimated from linkage maps, it seems unlikely that recombination rate is greatly increased in the females of domestic mammals.

Although linkage maps provide both female and male broad-scale recombination rate estimates, there are instances in which the number of MLH1 foci (reflecting only recombination in males) might be the only way to obtain such estimates, even when access to spermatocytes might be complicated. The construction of genetic linkage maps requires access to both an extensive number of markers that provide a good coverage of the genome and large known pedigrees. While the former is becoming less challenging with current developments in genomic technology, large pedigrees of wild species are rare. In addition, estimates of broad-scale recombination rates based on the number of MLH1 foci might be preferred to estimates based on linkage maps. The resolution of maps is compromised by marker coverage, in particular for the telomeres, which may lead to overestimate the sex-differences in recombination rate (Coop and Przeworski 2007); for example, in humans and other placental mammals, males recombine more towards the telomeres while females have higher recombination rates near the centromeres. In addition, linkage mapping is based on transmitted chromosomes, and thus provide no information about half the crossovers that occur in meiosis or about gametes that may be selected against (Vallente et al. 2006).

Recombination around genes associated with phenotypic characters in dogs

Direct methods, such as counting MLH1 foci or sperm-typing studies, provide a contemporary measure of recombination rate, but may not be fully informative about historical recombination at the population level. In humans, significant discrepancies have been found between sperm crossover frequencies and historical recombination rates at specific sites, which have been attributed to the rapid evolution of hotspots and their transient activity (Jeffreys and Neumann 2005; Jeffreys and Neumann 2009). While our results above indicate that recombination rate may not have changed at the genome-wide level during domestication, it is possible that artificial selection may have favored individuals with increased recombination around loci associated with selected phenotypes, so that these genes would be decoupled from surrounding regions, making artificial selection more efficient. Our results showed, for the 16 genomic

regions studied, no difference in the overall number of recombination events across dogs and wolves (equality of the slopes, Fig. 2). Likewise, we found no differences in the proportion of peaks with higher-than-average recombination rate in central vs. flanking windows in dogs as compared to wolves.

LDhat analyses provide population recombination rate estimates. We did not attempt to identify hotspots or compare the intensity of recombination between wolves and dogs, and thus we did not re-scale the population recombination rate, $\rho = 4Ner$, to per-generation recombination rate, r (measured in cM/Mb) using the effective population size, *N*e, of dogs or wolves. Indeed, great uncertainty surrounds *N*e estimates for these species. Axelsson et al. (2012) and Auton et al. (2013) obtained estimates of *N*e for dogs that differed 4-fold, and Freedman et al. (2014) detected very large changes in effective population size since the time of domestication and in different wolf lineages. Although demography and selection are confounders of recombination rate (see Pritchard and Przeworski 2001; Clark et al. 2010 and Chan et al. 2012 for excellent reviews), here we assess the distribution of recombination breakpoints along the regions for each species or population, and thus differences in demography and selection across species or populations should not bias our results. Even if the power to detect recombination events were not the same in all groups of samples, the recombination events detected did not tend to accumulate in the center of the chromosomal region under study in dogs more than in wolves. Consequently, our results do not support an increase recombination in these regions in dogs.

Chan et al. (2012) indicated that LDhat may spuriously detect hotspots in the presence of a selective sweep. In this respect, our results are conservative, because the bias in LDhat should lead to a higher number of hotspots toward the center of the chromosomal regions under study in dogs, where the loci under selection are located, and we observed no differences between the two species.

Domestication and changes in the recombination rate

Phylogenetic relationships (Dumont and Payseur 2008) as well as the number of chromosome arms (Pardo-Manuel de Villena and Sapienza 2001) have been shown to have an effect on recombination rates. In this study, we included species pairs separated by small phylogenetic distance; the dog was compared with its direct wild ancestor, the gray wolf (Vilà et al. 1997) and, in the case of the sheep and the goat, for which the wild ancestors are less clear (Bruford and Townsend 2006; Luikart et al. 2006) and the candidate species are in most cases vulnerable or threatened, we chose to work with a closely related wild congener. In addition, the two species being compared had equal chromosome number and organization (meta- or acrocentric).

Ross-Ibarra (2004) distinguished between wild progenitors and congeners to test both the Rees and Dale (1974) hypothesis for an increase in recombination rate in domestic species, as well as the Gornall (1983) hypothesis of preadaptation, in which higher recombination rate would predate, and contribute, to success in domestication. Our results do not support the hypothesis that domestic animals have higher recombination rate than their wild counterparts. Although only three pairs of species were compared, they represent early domesticates, with large diversity and world-wide distribution, and all of them showed higher recombination rate in the wild species. In addition, given that the domestic species we investigated had an average number of crossover markers close to the minimum expected for the correct segregation of chromosomes, we find that the preadaptation hypothesis is not likely either. Ross-Ibarra (2004) did not find support for the pre-adaptation hypothesis in plants, and concluded that increased recombination rate was of little importance in the process of domestication. Our results show that domestication may have not been associated with an increase in the recombination rate in mammals, even though the Burt and Bell (1987) study is often cited as an example (see Introduction). This study did not include wild progenitors of the domestic species and, therefore, it was inconclusive in assessing whether domestication resulted in an increased recombination rate (Ross-Ibarra 2004; Coop and Przeworski 2007).

Even if broad-scale recombination rates had not changed, it was possible that strong artificial selection may have contributed to increased recombination around loci associated to distinct phenotypes, so that these genes would be decoupled from surrounding regions, making artificial selection more efficient. Our results in dogs and gray wolves showed no evidence for differences in the number and distribution of recombination breakpoints in 16 genomic regions (200-300 Kbp) around loci potentially associated to phenotypes subject to strong selection in dogs. In these species, even for purebred dogs, linkage disequilibrium decay is rapid over less than 50 kb, and is very limited over 100 kb (Lindblad-Toh et al. 2005; Axelsson et al. 2012). Thus, the length of the regions studied seems adequate to detect changes in recombination rate. Although these results may reflect genome-wide patterns, due to the small number of regions studied here of a given length, a more conclusive confirmation of the results may require individual recombination maps (see below) on a larger number of samples.

An alternative prediction proposed that selection for reduced recombination in domesticated species may protect from maladaptive gene flow from wild relatives (Lenormand and Otto 2000). For the three pairs of species studied here, the overall recombination rate is lower for the domestic counterpart, apparently supporting this hypothesis. Nevertheless, it is not clear if this process should affect all domestic mammals, since many of them spread far beyond the distribution range of the ancestor species soon after domestication (sheep and goat, for example; for other species, the wild ancestor became extinct, as is the case of the horse), thus decreasing the chances for intercrossing and the possible selection for lower recombination rate.

Based on the results presented in this paper, we find no support for the idea that strong directional selection resulted in the evolution of increased recombination rate in domestic mammals, or that increased recombination associated to selected loci during domestication facilitated a response to selection. It has been proposed that rates of recombination may evolve neutrally, with selection pushing them back to the neutral range if they drift towards low or high

recombination rates (Dumont and Payseur 2008).

Current advances in genome sequencing allow massive parallel whole-genome amplification of single sperm cells followed by high-throughput genotyping to construct an individual's recombination map (Lu et al. 2012; Wang et al. 2012; Kirkness et al. 2013). Phased SNPs or haplotypes are obtained, which enable recombination events and possibly also gene conversion events to be directly identified, and individual high-resolution maps to be built, irrespective of a pre-selection of candidate genes or loci as we have done in this study. While sperm cells might be obtained in large numbers from adult males, via ejaculation or from dead animals (e.g. Muñoz-Fuentes et al. 2014), applying this technique to oocytes remains challenging, mainly due to the temporal aspects of mammalian oogenesis. Thus, these studies, like previous ones, may be potentially limited to the more readily available male samples. Applying this technique to dogs and wolves will be particularly interesting as, like other canids analyzed (Muñoz-Fuentes et al. 2011; Axelsson et al. 2012), they seem to lack a functional PRDM9. So far, it seems that PRDM9 in *Ovis* sp and *Capra* sp has not been investigated.

Materials and Methods

Cytology and immunofluorescence assays

Samples. Testes from dogs, wolves, pigs, wild boar, sheep, mouflons, goats and ibexes were opportunistically collected and tissue samples snap frozen in liquid nitrogen. All samples used in immunofluorescence analyses were obtained in Spain and were available for reasons other than this study. We contacted veterinary clinics for the dog samples (derived from castration), zoos for the wolf samples (from dead wolves), slaughterhouses for pig, sheep and goat samples, and attended hunting events to collect wild boar, mouflon and ibex samples.

We obtained high quality cell preparations for spermatocyte spreads against MLH1 from 6 dogs, 2 wolves, 6 goats, 6 ibexes, 6 sheep and 5 mouflons (Table S1). In addition, these experiments were also carried out for pig (*Sus domesticus*) and wild boar (*S. scrofa*) spermatocytes (7 and 10 individuals, respectively), but we were unable to visualize MLH1 proteins. Since these samples were collected as the others and were preserved and processed in the same way, we attribute the lack of success to the antibodies not recognizing pig and wild boar MLH1 proteins.

Immunofluorescent localization of MLH1 protein on spermatocytes synaptonemal complexes. Spreading and immunostaining of spermatocytes was performed as in Roig et al. (2004). Briefly, a piece of frozen testis was minced in PBS to obtain a cell suspension. Cell membrane was disrupted with the addition of 1% Lipsol (DH Scientific) (diluted in water) and incubating at 4ºC for approximately 14 minutes. Cells were fixed on slides for 2 hours with 1% paraformaldehyde, 0.15% Triton X-100 and 1x protease inhibitor cocktail (Roche) in water. Slides were rinsed in 1% Photo-Flo solution (Kodak) and blocked with 0.2% BSA, 0.2% Gelatin, 0.05% Tween-20 in PBS (PTBG). Incubation of rabbit polyclonal antibody against SYCP3 (dilution 1:200, Abcam) to mark chromosomes and mouse monoclonal antibody against MLH1 (dilution 1:50, Pharmingen) was performed at 4ºC overnight. After 4 washes with PTBG, CY3-conjugated antibody against rabbit and an FITC-conjugated antibody against mouse antibodies (dilution 1:100, Jackson Immunoresearch) were incubated 1h at 37ºC. Slides were then washed four times in PTBG and DNA counterstained with DAPI dissolved in Vectashield mounting medium (Vector Lab).

Slides were analyzed using a Zeiss Axioskop fluorescence microscope. Only well spread cells displaying bright foci were captured and processed by Progress Capture software (Jenoptik). Images were further enhanced using Adobe Photoshop version CS2 to match the fluorescent intensity seen in the microscope. To avoid biases, for a subset of samples, MLH1 foci were counted by at least two investigators. In all these cases, similar results were obtained by the

different researchers (data not shown). Furthermore, the same person counted the foci in each domestic and wild species pair. Foci were counted in 14-75 spermatocytes per individual.

To investigate the variation in the number of MLH1 foci, we used the generalized linear mixed model function in R ver. 3.0.3 (R Core Team 2014) "lmer()" in the package "lme4" (Bates et al. 2014). We set the number of foci per cell as the dependent variable, species as fixed factor (wild or domestic), and individual as random factor. Assuming a normal error distribution, the model fitted the data well, with the residuals following a straight line in a normal probability plot (QQ plot). To compute *P* values, we used the function "cftest()" in the package "multcomp" (Hothorn et al. 2008).

Population genomic inferences of recombination

Sample collection and sequencing. Mouth swab samples were collected from 5 mongrel dogs (dogs of unknown ancestry, except one pure breed German shepherd) and blood or tissue samples from 22 dead wolves from widespread geographic locations (British Columbia, Canada, *n*=2; Finland, *n*=3; Italy, *n*=3; North Western Territories, Canada, *n*=3; Spain, *n*=4; Sweden, *n*=4; United States, *n*=2) (Table S1). Mongrel dogs were preferentially analyzed for two reasons; first, to increase the number of polymorphisms (SNPs) per individual, which would increase the power to detect recombination, and, second, to avoid biases that could be associated with certain breeds, as the rate of recombination is a heritable trait and inbreeding could lead to interbreed differences. Except for the wolves from the United States, which were captive and from which blood samples were obtained, all other wolves were wild and died for reasons unrelated to this study.

We extracted DNA using the QIAgen DNeasy kit and prepared Illumina paired-end libraries for each sample using the Agilent protocol for indexed paired-end Illumina libraries and Agilent SureSelect capture system. Briefly, DNA was sheared with a Covaris S2 device (Covaris, Inc.

Woburn, MA, USA), end-repaired, A-tailed, ligated with Illumina's indexing-specific paired-end adaptors and PCR amplified for 5 cycles.

We enriched for 16 chromosomal regions, each containing a locus associated with a distinct phenotypic character (morphological or behavioral) in dogs (Table 1) in a central position, and 100-150 kb upstream and downstream (totalling ca. 200-300 kb in length). We enriched with a custom Agilent SureSelect RNA oligo kit. The oligo-targeted regions added up to 2.48 Mbp (repetitive regions excluded) and encompassed approximately 3.96 Mbp of the dog genome. Libraries were then Illumina indexed/barcoded in a PCR of 13 cycles. A Nanodrop spectrophotometer and a Bioanalyzer instrument were used to assess both quality and quantity of the samples at various steps during the laboratory procedures. Libraries were validated using real-time quantitative PCR, pooled and then 90 or 100-bp paired-end sequenced on 4 lanes of an Illumina GAIIx machine, yielding 84,682,789 of paired-end reads.

Alignment of reads and SNP calling. We followed the Broad Institute Best Practice Variant Detection guide (http://www.broadinstitute.org/gatk/guide/topic?name=best-practices) for data processing and analysis. Briefly, we aligned raw reads using BWA 0.6.1-r104 (Li and Durbin 2009) at 4 edit distance to the CanFam2 reference assembly downloaded from the UCSC (University of California Santa Cruz) Genome Browser. PICARD TOOLS 1.66 (http://picard.sourceforge.net) and SAMTOOLS 0.1.18 (Li et al. 2009) were used to remove PCR duplicates and multimapping reads, respectively, and at various stages during the mapping and SNP calling procedures to manage files. We used the Genome Analysis Toolkit (GATK) 2.1.9 (McKenna et al. 2010) to realign around indels, perform base quality recalibration, call SNPs using UnifiedGenotyper, and then filter using VariantFiltration to avoid false-positive SNP calls (DePristo et al. 2011). We excluded indels and filtered variants following Auton et al. (2012) with some modifications. We used BEDTOOLS (Quinlan and Hall 2010) to extract information at various stages during the bioinformatic procedures. On average, we mapped 97% of the reads per sample, which was reduced to 79% after removing PCR duplicates and

multimapping reads. The proportion of reads on target was 40-60%. Our filtered SNP set consisted of 22,614 SNPs, of which 17,390 were typed in all individuals. Data were phased using BEAGLE (Browning and Browning 2007).

Population-genetic inferences of recombination. As a measure of linkage disequilibrium (LD), or population-level non-random association of alleles at two loci, we used the r^2 statistic. It ranges from 0 to 1, and it equals 0 when the two alleles are in equilibrium, that is, the loci are independent of one another. We calculated r^2 on the phased data using VCFTOOLS 0.1.10 (Danecek et al. 2011) and constructed LD maps, in which pairwise LD measures are plotted between each pair of SNPs, using the R function "LDheatmap()" (Shin et al. 2006). Wolf genotype data was previously thinned to match the dog data in the number and location of SNPs, by selecting the SNP with the same or the closest coordinates to each dog SNP.

Representations such as LD maps based on r^2 allow the identification of haplotype blocks, but they do not allow us to directly associate differences in patterns between a pair of SNPs with differences in the underlying recombination rate. In order to characterize the non-random association of alleles in the population due to recombination, methods have been developed to statistically determine recombination breakpoints or to estimate the likelihood of the observed sample data under population models that assume different sets of population genetic parameters (e.g., recombination rate, mutation rate) and that attempt to include all the information present in the data through the underlying genealogy (Posada et al. 2002; Stumpf and McVean 2003). The latter is generally computationally intractable for large data sets using full-likelihood methods and, in this respect, an important contribution has been the development of approximate-likelihood methods to infer the population recombination rate, $rho = 4N_e r$, from a large number of markers (McVean et al. 2004; Auton and McVean 2007).

We used RDP, GENECONV, BOOTSCAN, MAXCHI, CHIMAERA, SISCAN and 3SEQ, as implemented in RDP3 (Martin et al. 2010), to simultaneously estimate the number of

recombination breakpoints. We also used LDhat 2.2 (McVean et al. 2004; Auton and McVean 2007), which implements a coalescent-based model to infer population recombination rates between adjacent SNPs. Because the wolves came from different populations, we performed the analyses for the wolves in separate groups according to continent of origin (North America, n = 7; Europe (Spain, Italy, Sweden and Finland), n = 14; and all together, n = 21). Due to computation limitations in RDP3, we further divided the wolves from Europe in two groups (Italy and Spain, and Finland and Sweden; n = 7 in each case) for those analyses.

We made input alignments files for RDP3 with a custom script and accepted breakpoints that were detected by at least two methods (Posada et al. 2002; Martin et al. 2010). We then compared the number of recombination breakpoints across regions for dogs and wolves.

In order to run the program *interval* as implemented in the LDhat package, we downloaded a lookup table for n = 50 sequences and a population mutation rate q = 0.001 per site from http://ldhat.sourceforge.net/instructions.shtml. We then generated adequate lookup tables for the number of sequences in our data set using the program *lkgen* from the LDhat package. Recombination rates were estimated with a block penalty of 5 and 10 million MCMC iterations, and we sampled from the chain every 5,000 iterations and discarded the first 100,000 as burn-in, following recommendations in the manual. Because no reliable estimates of effective population size were available for dogs and wolves (Axelsson et al. 2012; Auton et al. 2013), we report only the estimates of the population recombination rate parameter as obtained with this method (see Discussion).

We then identified the number of **h**igher **t**han **a**verage **r**ecombination peaks (HTAR peaks; number of regions with recombination rate above the average as inferred by LDhat, indicated by a dashed line in Fig. 3 and Fig. S3) along each of the 16 genomic regions in three windows of 70 kb in size at the two ends of the region, and a central window of size between 60 and 160 kb. The central window length varied according to the length of the chromosomal region captured,

and was longer in the cases in which the locus associated with the dog trait was larger (haplotype instead of a point mutation) (Table 1). However, since the size of the fragments in dogs and wolves were equal in size, no bias was introduced in this respect in the comparisons. We used a Fisher's exact probability test to compare the ratios of HTAR peaks in central to flanking windows in dogs and North America wolves, dogs and European wolves and dogs and all (North American and European) wolves (16 x 3 = 48 comparisons performed).

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Author contributions:

VMF, CV, AD, IR and AT designed research; VMF, CLF, JMM, EMP, AS and CV collected samples; VMF, MMO, GAA, KD and AV performed experiments; VMF, CV, IR and AT analyzed the data; VMF and CV wrote the first draft of the paper. All authors revised the manuscript and contributed to the representation of results.

The authors declare no conflict of interest

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Figure Legends

Figure 1. Spermatocytes from wild mammals have more crossover markers than cells from domestic mammals. Spermatocytes from dog (A), wolf (B), goat (D), ibex (E), sheep (G) and mouflon (H) immunostained against the crossover marker MLH1 (red) and the synaptonemal complex protein SYCP3 (green), which allows to visualize chromosome axes. DNA is counter-stained with DAPI (blue). Scatterplots display the total number of MLH1 foci found in each spermatocyte, black lines represent the average number of foci found in each species (C, F and I). Domestic species, green; wild species, red.

Figure 2. Number of segregating sites and recombination breakpoints per chromosome for the 16 genomic regions studied in dogs and wolves. Each dot represents the number of historical recombination breakpoints inferred in a particular genomic region and the number of segregating sites found in that region.

Figure 3. Genetic maps for dogs and wolves. Genomic region 1 (Table 1) is shown here (see Fig. S3 for all regions). Mean $\rho = 4N_e r$ estimates along each fragment (penalty 5). Each dot represents the value of ρ between each pair of SNPs. The color of the dot indicates whether the SNP is found in an intergenic region (green), an intron (pink) or an exon (blue, synonymous change; red, non-synonymous change). The red line represents the location of the locus associated with the phenotypic character (see Table 1). The dashed line is the average recombination rate for the region.

Supporting Figure Legends

Supporting Figure 1. MLH1 foci counts for all samples. MLH1 counts for all individuals analyzed: dogs and wolves (green and red, respectively, A), goats and ibexes (green and red, B) and sheep and mouflons (green and red, C). Black lines represent the average number of MLH1 foci counted in each individual.

Supporting Figure 2. LD patterns in dogs and wolves for each of the regions studied. Values of r^2 as a measure of pairwise LD between SNPs. Wolf genotype data was thinned to match the dog data by selecting the wolf SNP with the same or the closest coordinates to each dog SNP. LDhat maps, based on the inference of population recombination rate (ρ), for the same markers, are plotted above each LD plot.

Supporting Figure 3. Genetic maps for the 16 genomic regions studied for dogs and wolves. Mean $\rho = 4N_e r$ estimates along each fragment (penalty 5). Each dot represents the value of ρ between each pair of SNPs. The color of the dot indicates whether the SNP is found in an intergenic region (green), an intron (pink) or an exon (blue, synonymous change; red, non-synonymous change). The red line represents the location of the locus associated with the phenotypic character (see Table 1). The dashed line is the average recombination rate for the region.

Supporting Table Legends

Supporting Table 1 Approaches and samples used in this study to compare recombination between wild and domestic species.

Supporting Table 2 Number of observed segregating sites and estimated number of recombination breakpoints in the 16 genomic regions studied in dogs and wolves.

Supporting Table 3 Mapping statistics of dog and wolf Illumina reads. Mapped and unmapped reads, multimapping reads and PCR duplicates.

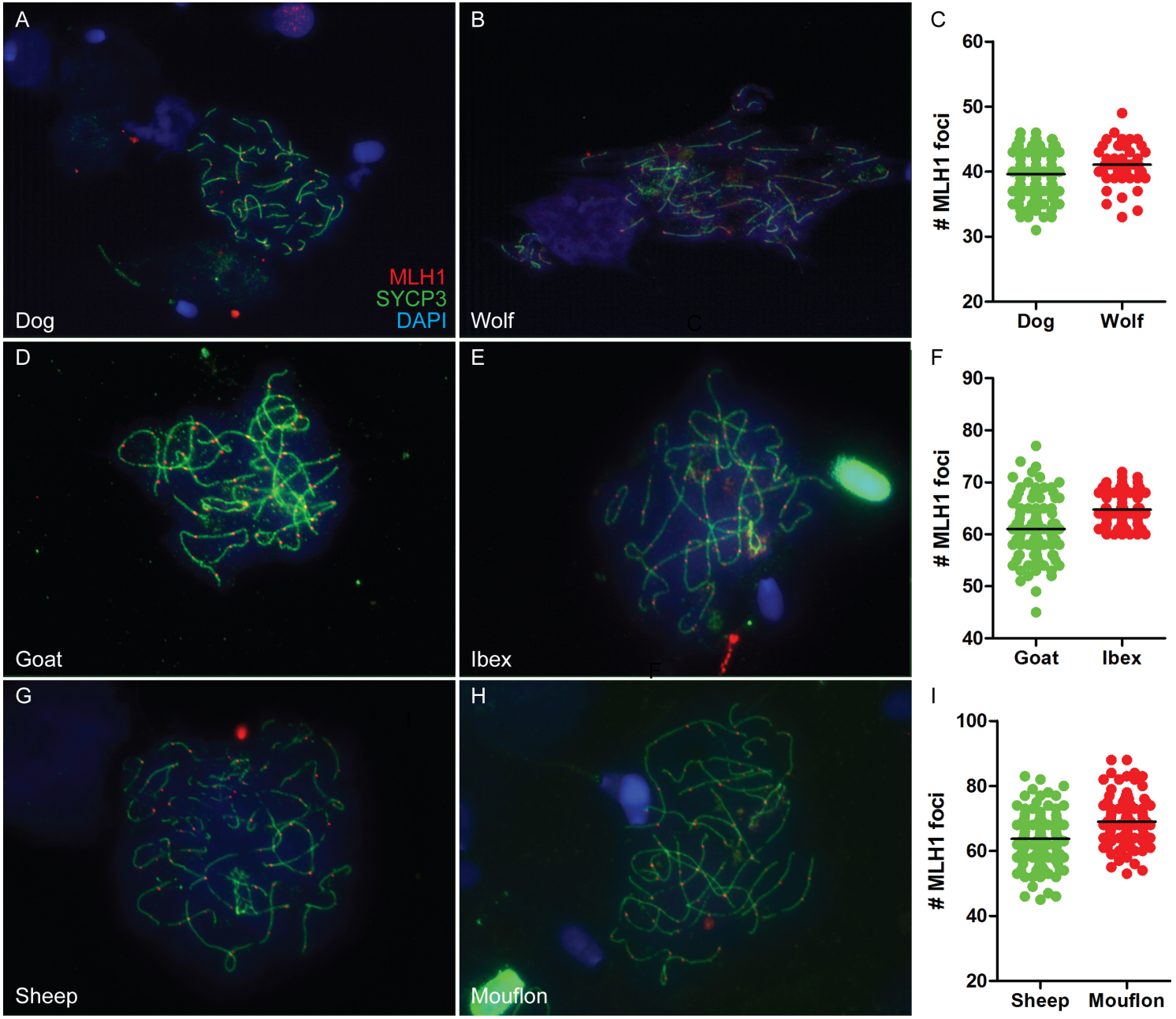
Table 1 Chromosomal regions studied, each containing centrally a locus associated to a distinct phenotypic character (morphological or behavioral) in dogs. Start and end refer to the CanFam2 assembly coordinates.

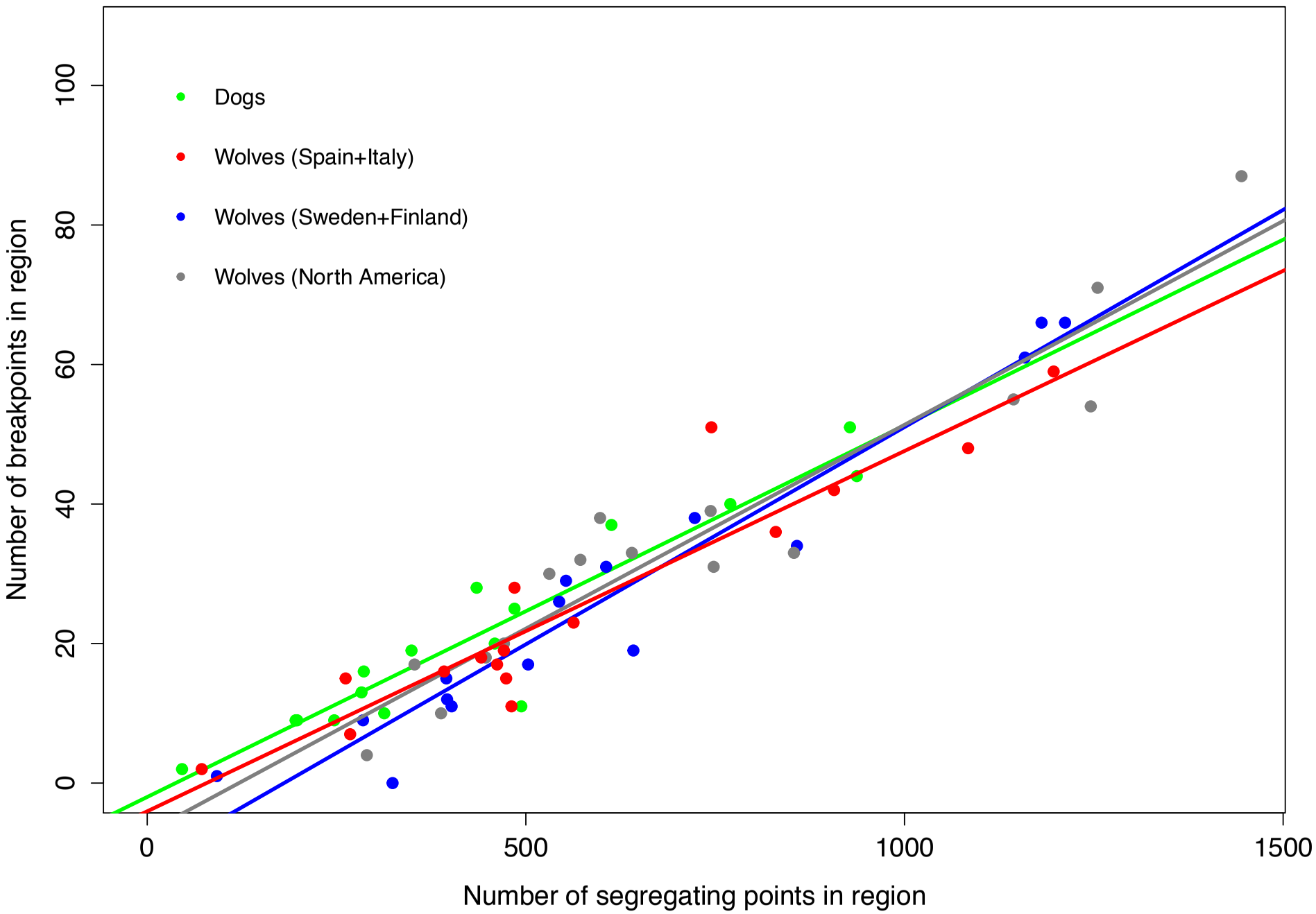
Chr	Target gene	Trait	Start	End
1	MC2R, C18orf1	Herding	27,318,228	27,521,819
9	STAT3	Neck ratio	23,799,353	24,030,794
10	SILV= PMEL	Merle coat	3,181,426	3,381,426
12	Runx2	Dorsoventral nose bend and midface length	16,637,470	16,946,083
13	RSPO2	Furnishings	11,484,766	11,784,766
15	IGF-1	Size	44,115,824	44,381,171
16a	LMBR1	"Dewclaw" or hind-limb-specific preaxial polydactyly	22,154,874	22,467,109
16b	K locus	Black coat	61,752,782	62,052,782
17	FOXI3	Lack of hair	40,996,789	41,197,329
18a	fgf4	Short legs	23,331,125	23,531,212
18b	FGF3 FGF4 FGF19	Hair ridge	51,298,518	51,631,941
20	M promoter of MITF	White spotting	24,747,309	25,049,039
22	PCDH9	Boldness	25,055,041	25,259,603
25	MLPH	Dilute coat	51,044,488	51,244,488
27	KRT71	Curly coat	5,442,806	5,642,806
32	FGF5	Coat length	7,373,337	7,573,337

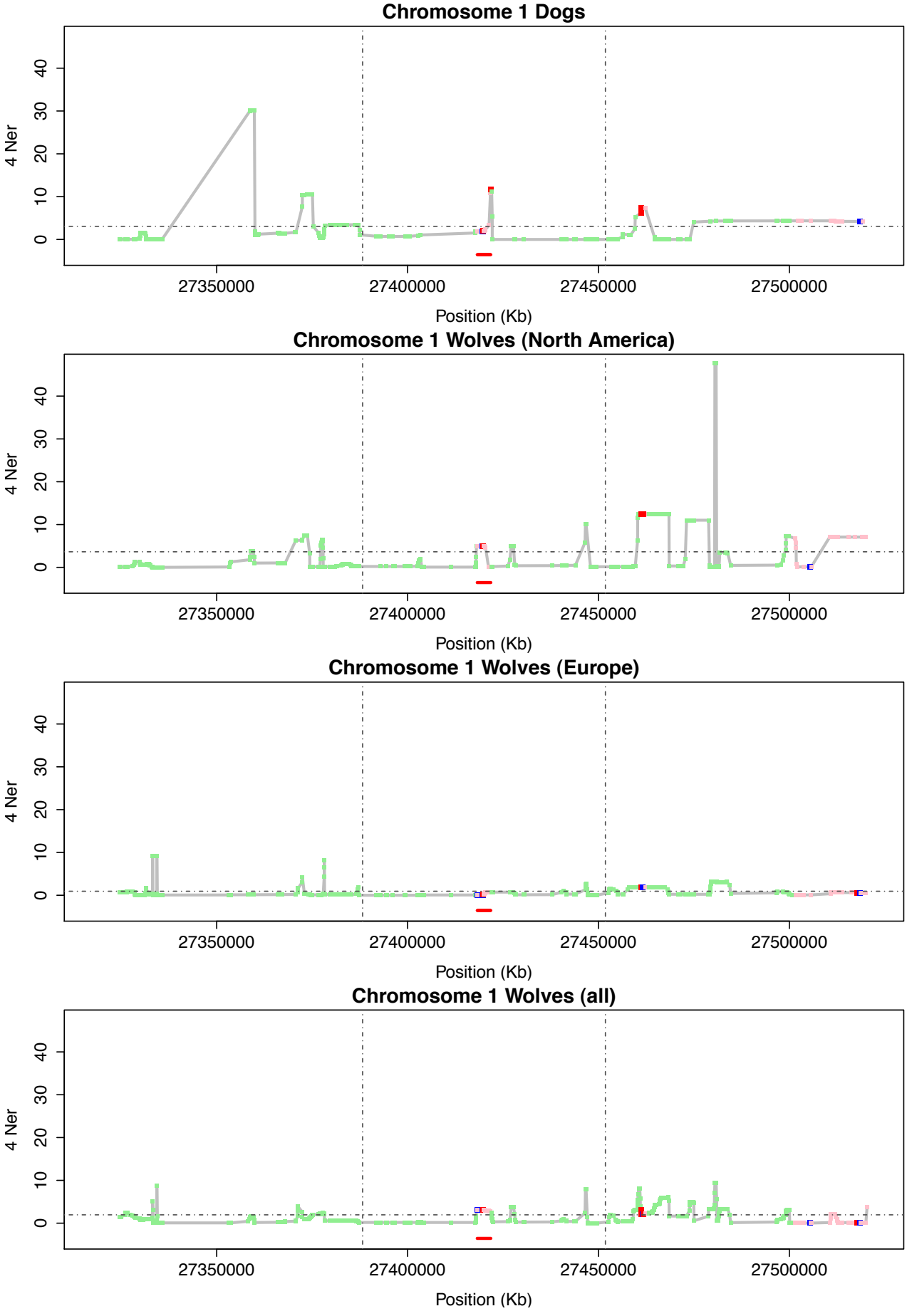
Table 2 Mean number of MLH1 foci per cell and standard deviation (SD) calculated over the individual means (means calculated over all measurements were almost identical, results not shown) and the estimated number of crossovers (COs) per chromosome pair. *N*, number of individuals; *n*, total number of spermatocytes for which MLH1 was counted.

Species	*N*	*n*	Mean number of MLH1 foci / cell	SD	Chromosome pairs	COs / chromosome pair	Chromosome type	COs / chromosome arm
Dog	6	184	38.89	0.87	39	1.00	acrocentric[1]	1.00
Wolf	2	45	40.94	1.61	39	1.05	acrocentric[1]	1.05
Goat	6	109	61.24	4.03	30	2.04	metacentric	1.02
Ibex	6	119	64.74	1.08	30	2.16	metacentric	1.08
Sheep	6	125	63.47	3.42	27	2.35	metacentric	1.18
Mouflon	5	113	69.03	2.49	27	2.56	metacentric	1.28

[1]All autosomes are acrocentric.







Supporting Information

Phenotypic diversity in domestic mammals does not result from an increased recombination rate

Muñoz-Fuentes, V.; Marcé-Ortega, M.; Alkorta-Aranburu, G.; Linde-Forsberg, C.; Morrell, J. M.; Manzano, E.; Södeberg, A.; Daniel, K.; Villalba, A.; Toth, A.; Di Rienzo, A.; Roig, I.; Vilà, C.

This section contains: **Supporting Figures**

Supporting Tables

Supporting Figure Legends

Figure S1. **MLH1 foci counts for all samples.** Graphs display MLH1 counts for all individuals analyzed: dogs and wolves (green and red, respectively, A), goats and ibexes (green and red, respectively, B) and sheep and mouflons (green and red, respectively, C). Black lines represent the average found in each sample (A-C).

Figure S2. LD patterns in dogs and wolves for each of the regions studied. Values of r^2 as a measure of pairwise LD between SNPs. Wolf genotype data was thinned to match the dog data by selecting the SNP with the same or the closest coordinates to each dog SNP. LDhat maps, based on the inference of population recombination rate (ρ), for the same markers, are plotted above each LD plot.

Figure S3. Genetic maps for the 16 genomic regions studied for dogs and wolves. Mean $\rho = 4N_e r$ estimates along each fragment (penalty 5). Each dot represents the value of ρ between each pair of SNPs. The colour of the dot indicates whether the SNP is found in an intergenic region (green), an intron (pink) or an exon (blue, synonymous change; red, non-synonymous change). The red line represents the location of the locus associated with the phenotypic character (see Table 1). The dashed line is the average recombination rate for the region.

Supporting Table Legends

Table S1 Approaches and samples used in this study to compare recombination between wild and domestic species.

Table S2 Number of observed segregating sites and estimated number of recombination breakpoints in the 16 genomic regions studied in dogs and wolves.

Supporting Tables

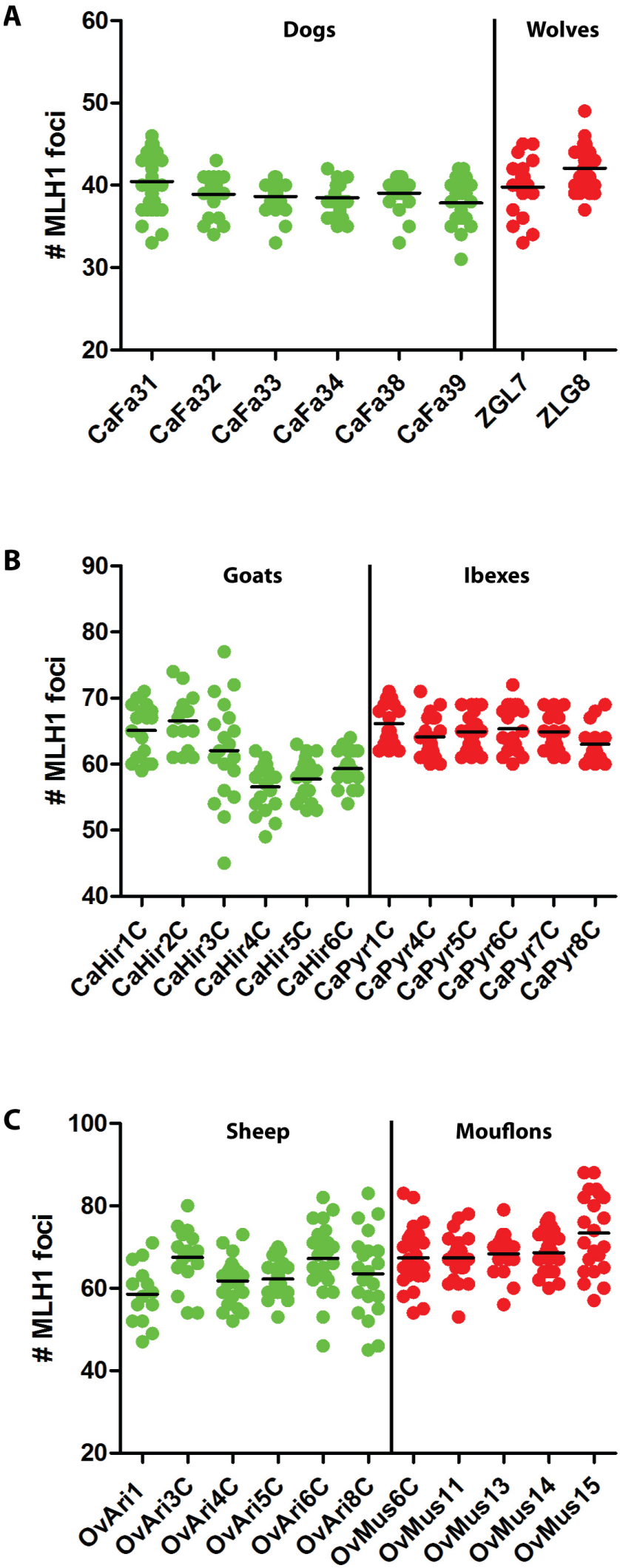
Table S1 Approaches and samples used in this study to compare recombination between wild and domestic species

Approach	Species	Common name	Origin	Sample ID
MLH1 mapping	*Canis familiaris*	Dog	Veterinary clinic	CaFa31
	Canis familiaris	Dog	Veterinary clinic	CaFa32
	Canis familiaris	Dog	Veterinary clinic	CaFa33
	Canis familiaris	Dog	Veterinary clinic	CaFa34
	Canis familiaris	Dog	Veterinary clinic	CaFa38
	Canis familiaris	Dog	Veterinary clinic	CaFa39
	Canis lupus	Wolf	Madrid Zoo	Z/LG8
	Canis lupus	Wolf	Madrid Zoo	Z/LG7
	Capra hircus	Goat	Pilas	CaHir1
	Capra hircus	Goat	Pilas	CaHir2
	Capra hircus	Goat	Pilas	CaHir3
	Capra hircus	Goat	Pilas	CaHir4
	Capra hircus	Goat	Pilas	CaHir5
	Capra hircus	Goat	Pilas	CaHir6
	Capra pyrenaica	Spanish ibex	Cazorla	CaPyr1
	Capra pyrenaica	Spanish ibex	Cazorla	CaPyr4
	Capra pyrenaica	Spanish ibex	Cazorla	CaPyr5
	Capra pyrenaica	Spanish ibex	Cazorla	CaPyr6
	Capra pyrenaica	Spanish ibex	Cazorla	CaPyr7
	Capra pyrenaica	Spanish ibex	Cazorla	CaPyr8
	Ovis aries	Sheep	Los Corrales	OvAri1
	Ovis aries	Sheep	Los Corrales	OvAri3
	Ovis aries	Sheep	Los Corrales	OvAri4
	Ovis aries	Sheep	Los Corrales	OvAri5
	Ovis aries	Sheep	Los Corrales	OvAri6
	Ovis aries	Sheep	Pilas	OvAri8
	Ovis musimon	Mouflon	Cazorla	OvMus6
	Ovis musimon	Mouflon	Cazalla de la Sierra	OvMus11
	Ovis musimon	Mouflon	Cazalla de la Sierra	OvMus13
	Ovis musimon	Mouflon	Cazalla de la Sierra	OvMus14
	Ovis musimon	Mouflon	Cazalla de la Sierra	OvMus15
Population genomics on sequence data	*Canis familiaris*	Dog	Veterinary clinic	CaFa1Sp
	Canis familiaris	Dog	Veterinary clinic	CaFa2Sp
	Canis familiaris	Dog	Veterinary clinic	CaFa5Sp
	Canis familiaris	Dog	Veterinary clinic	CaFa8Sp
	Canis familiaris	Dog	Veterinary clinic	CaFa9Sp
	Canis lupus	Wolf	United States (captive)	465Bl
	Canis lupus	Wolf	United States (captive)	475Bl

	Canis lupus	Wolf	Spain	CaLu3M
	Canis lupus	Wolf	Spain	CaLu8M
	Canis lupus	Wolf	Spain	CaLu9M
	Canis lupus	Wolf	Spain	CaLu10M
	Canis lupus	Wolf	Sweden	CaLu4M
	Canis lupus	Wolf	Sweden	CaLu5M
	Canis lupus	Wolf	Sweden	CaLu6M
	Canis lupus	Wolf	Sweden	CaLu7M
	Canis lupus	Wolf	Finland	FIN7462
	Canis lupus	Wolf	Finland	FIN7760
	Canis lupus	Wolf	Finland	FIN8174
	Canis lupus	Wolf	Italy	IT001
	Canis lupus	Wolf	Italy	IT050
	Canis lupus	Wolf	Italy	IT973
	Canis lupus	Wolf	North Western Territories	NRS2010.005
	Canis lupus	Wolf	North Western Territories	NRS2010.016
	Canis lupus	Wolf	North Western Territories	NRS2010.021
	Canis lupus	Wolf	British Columbia	ex111
	Canis lupus	Wolf	British Columbia	ex112

Table S2 Number of observed segregating sites and estimated number of recombination breakpoints in the 16 genomic regions studied in dogs and wolves.

	Dogs		Spain + Italy wolves		Sweden+Finland wolves		North American wolves	
chr	**Segr. Sites**	**Breakpoints**	**Segr. Sites**	**Breakpoints**	**Segr. Sites**	**Breakpoints**	**Segr. Sites**	**Breakpoints**
1	247	9	268	7	324	0	353	17
9	198	9	392	16	402	11	388	10
10	46	2	72	2	92	1	290	4
12	485	25	563	23	642	19	854	33
13	494	11	481	11	606	31	748	31
15	196	9	262	15	285	9	447	18
16a	283	13	474	15	396	12	471	20
16b	928	51	907	42	1181	66	1255	71
17	435	28	485	28	503	17	598	38
18a	313	10	462	17	544	26	531	30
18b	937	44	1197	59	1212	66	1246	54
20	459	20	745	51	723	38	744	39
22	349	19	441	18	553	29	640	33
25	613	37	830	36	858	34	1144	55
27	770	40	1084	48	1159	61	1445	87
32	286	16	471	19	395	15	572	32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Wolves
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50
40
30
20
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ZGL7
ZLG8
B
Goats
Ibexes
MLH1 foci
90
80
70
60
50
40
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CaHir2C
CaHir3C
CaHir4C
CaHir5C
CaHir6C
CaPyr1C
CaPyr4C
CaPyr5C
CaPyr6C
CaPyr7C
CaPyr8C
C
Sheep
Mouflons
MLH1 foci
100
80
60
40
20
OvAri1
OvAri3C
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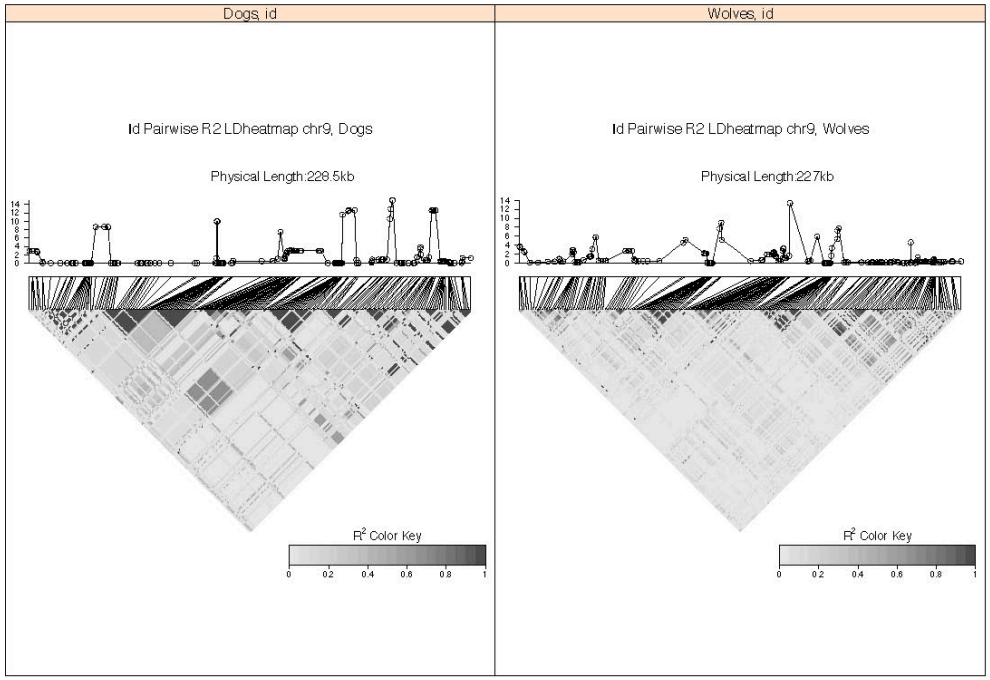
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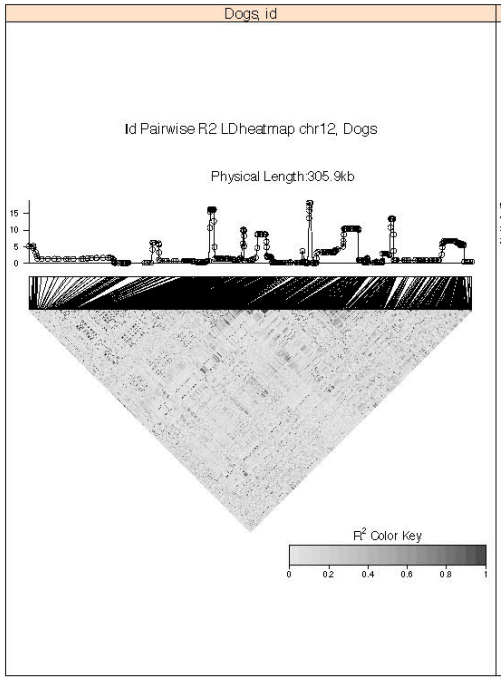
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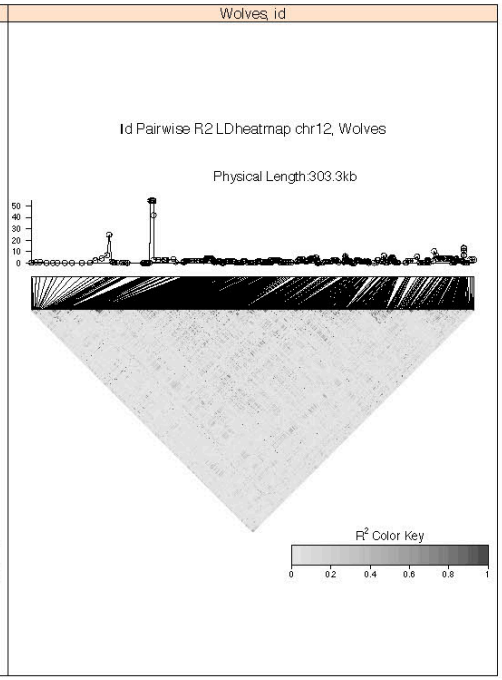
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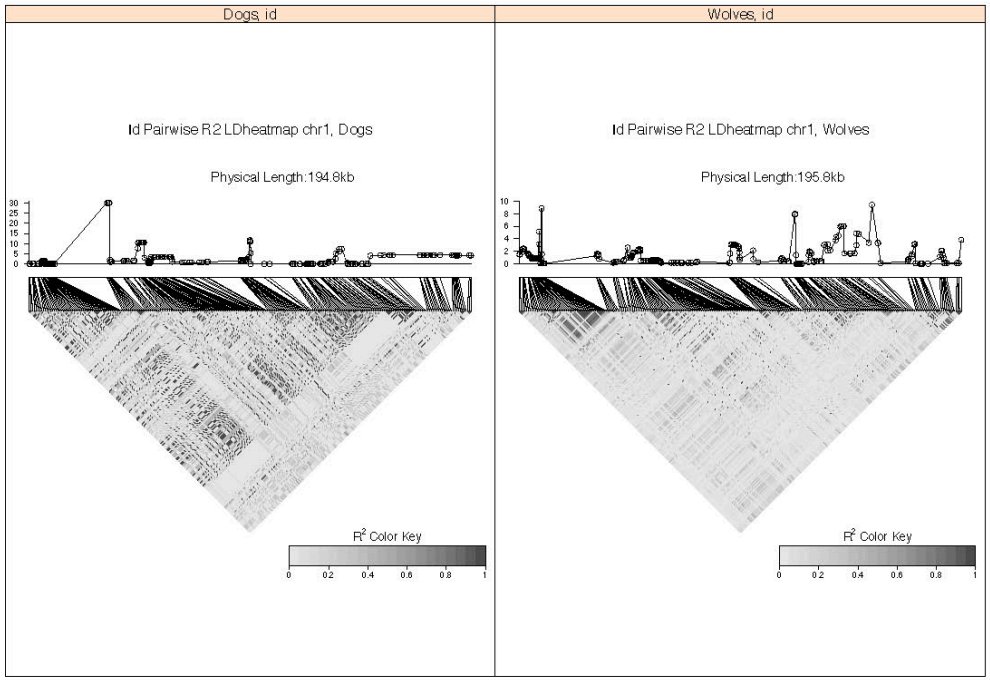
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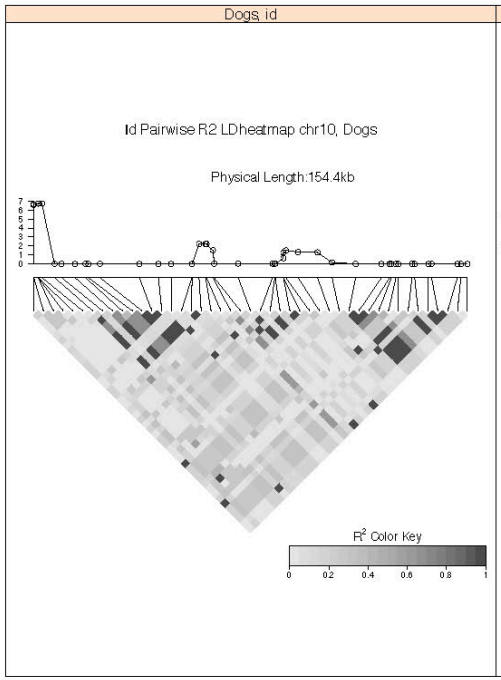
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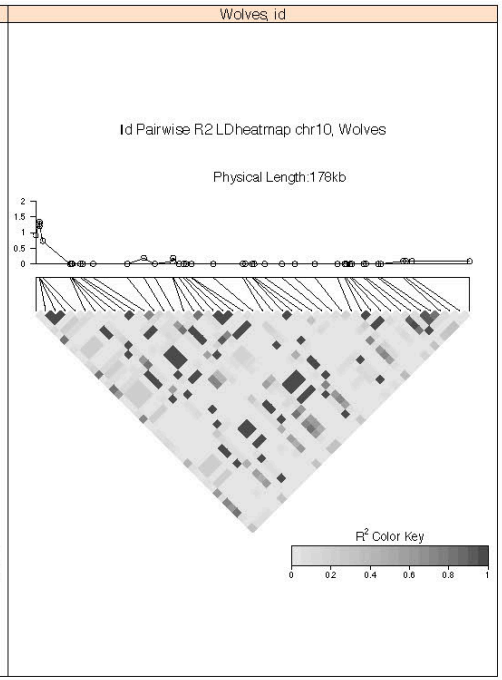
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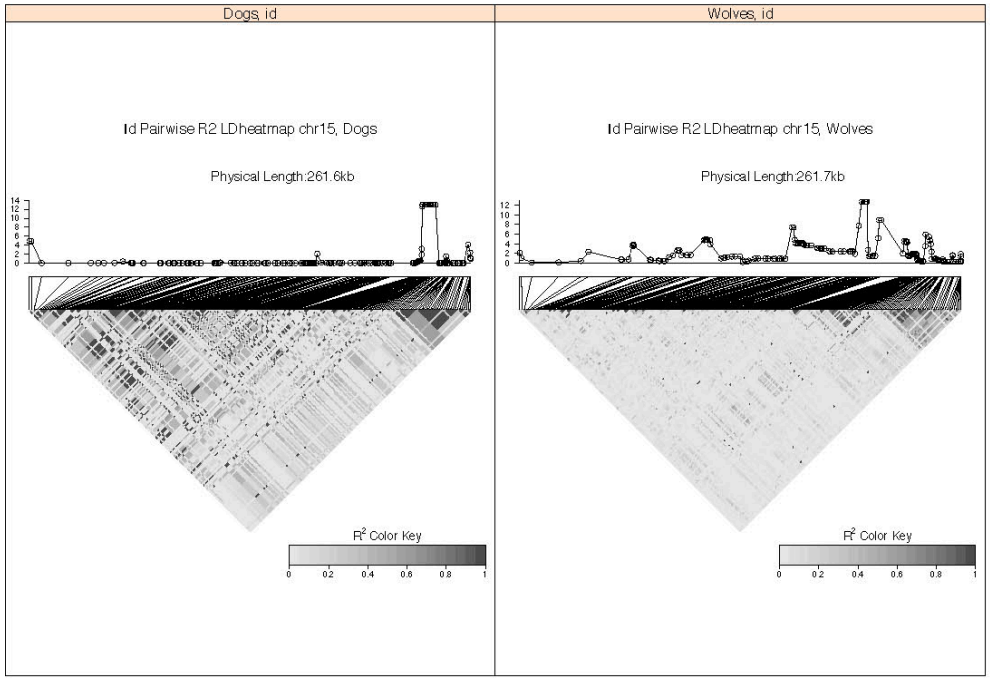
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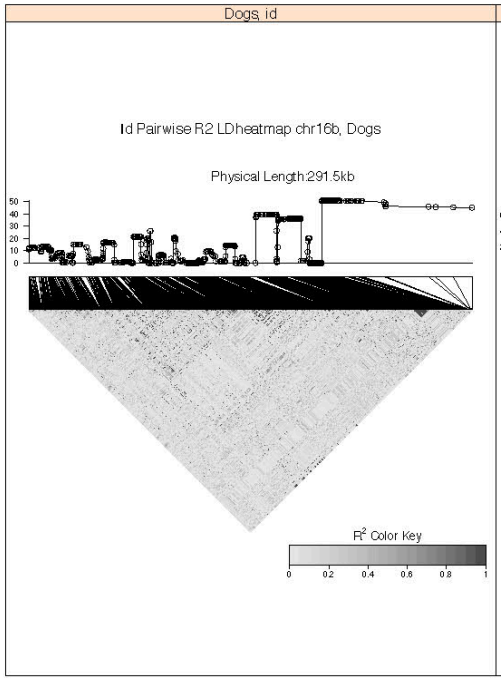
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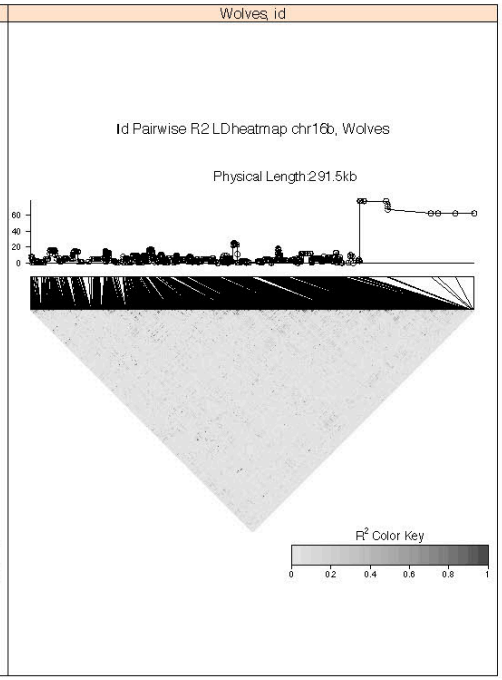
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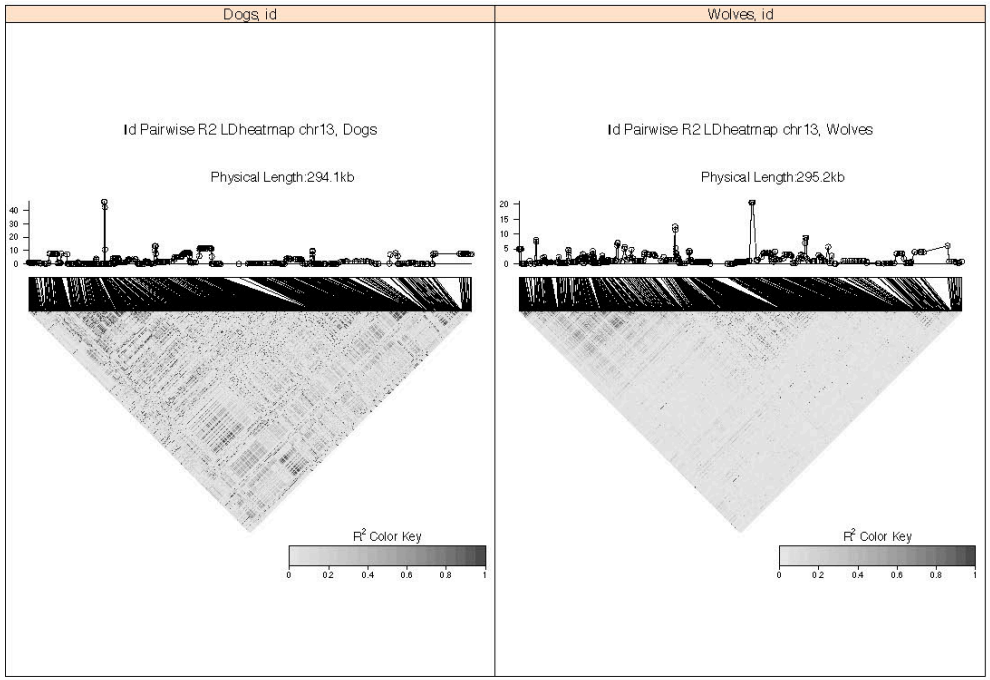
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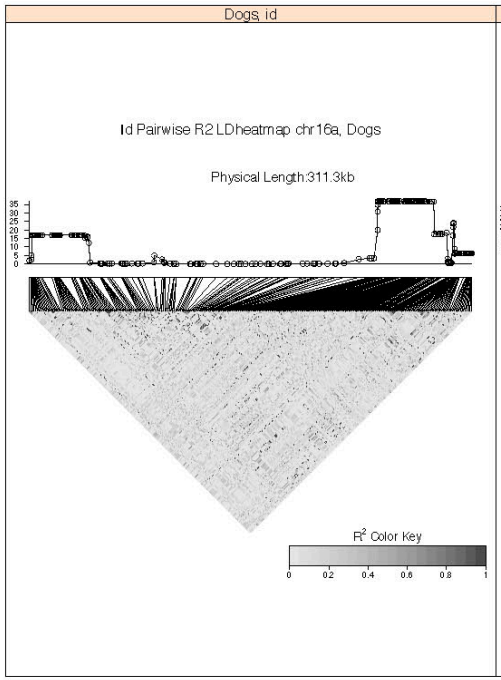
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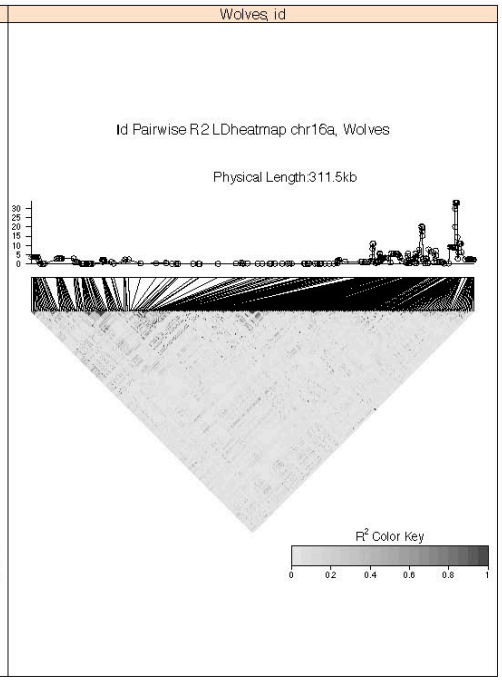
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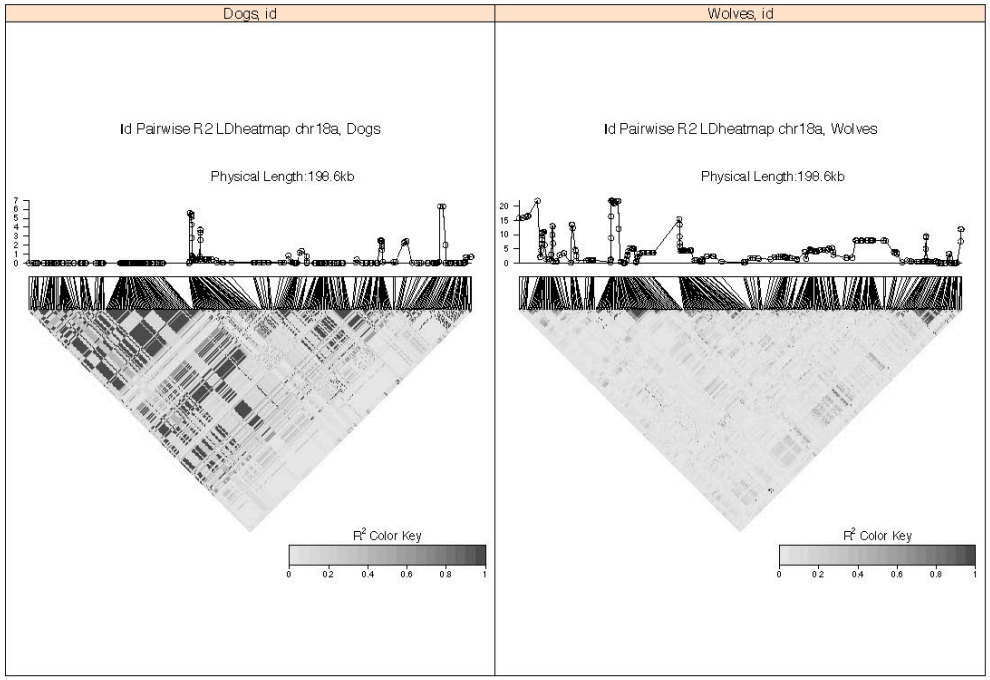
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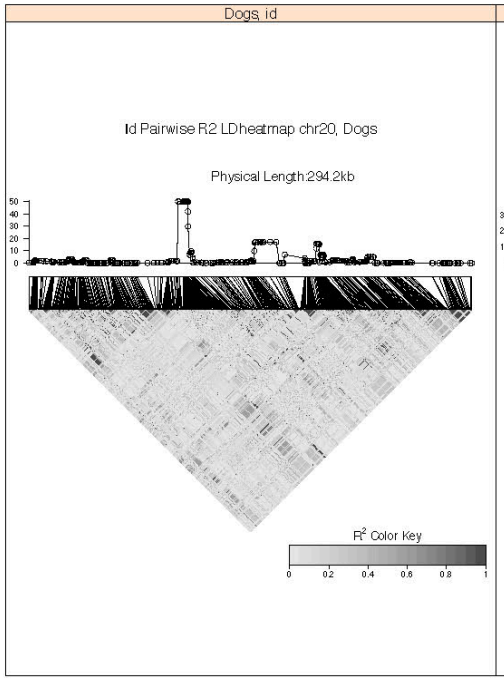
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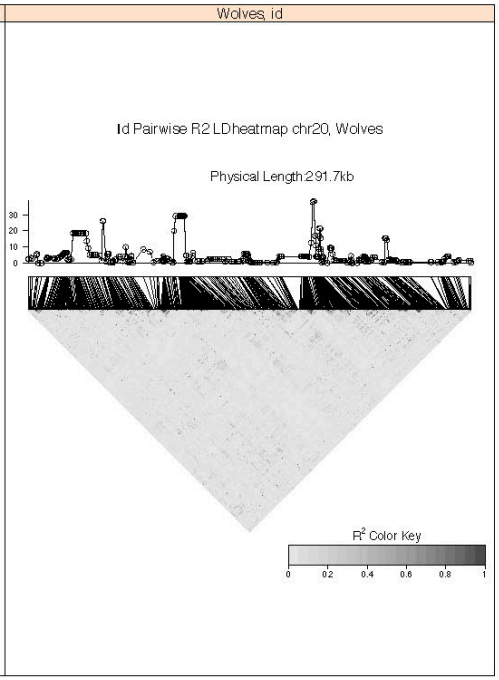
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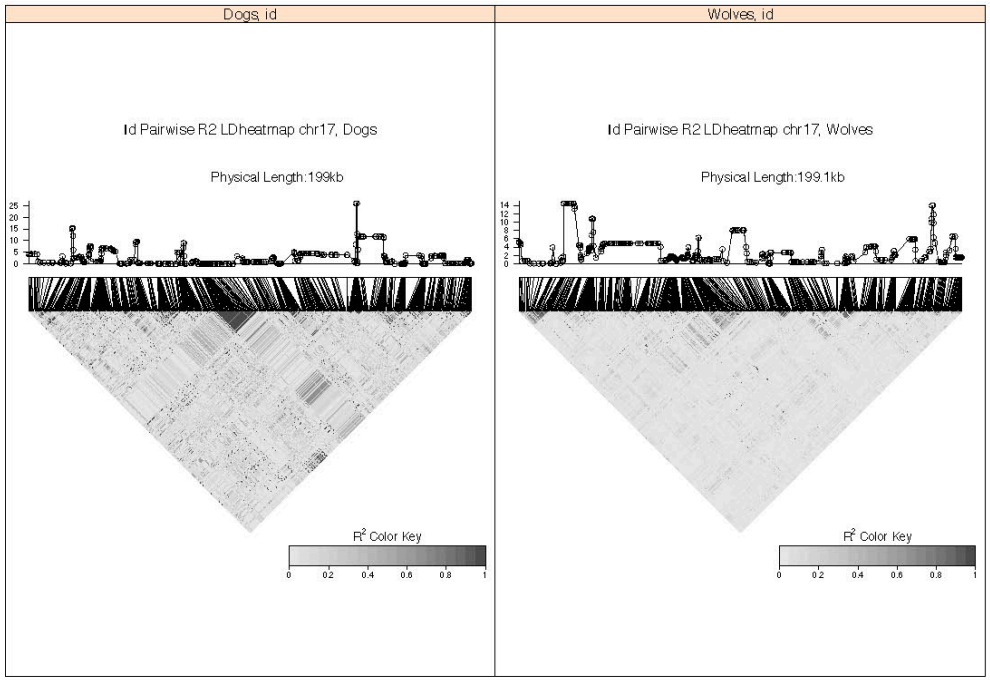
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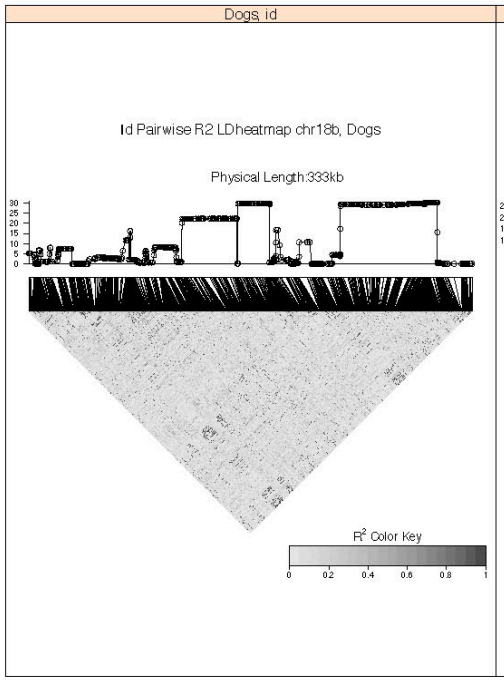
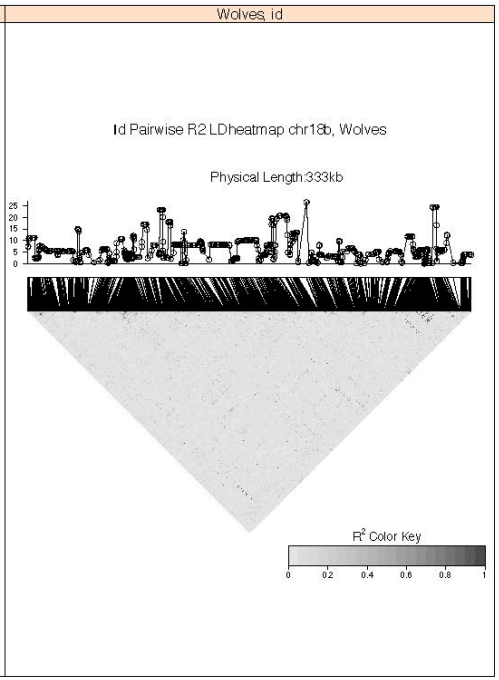
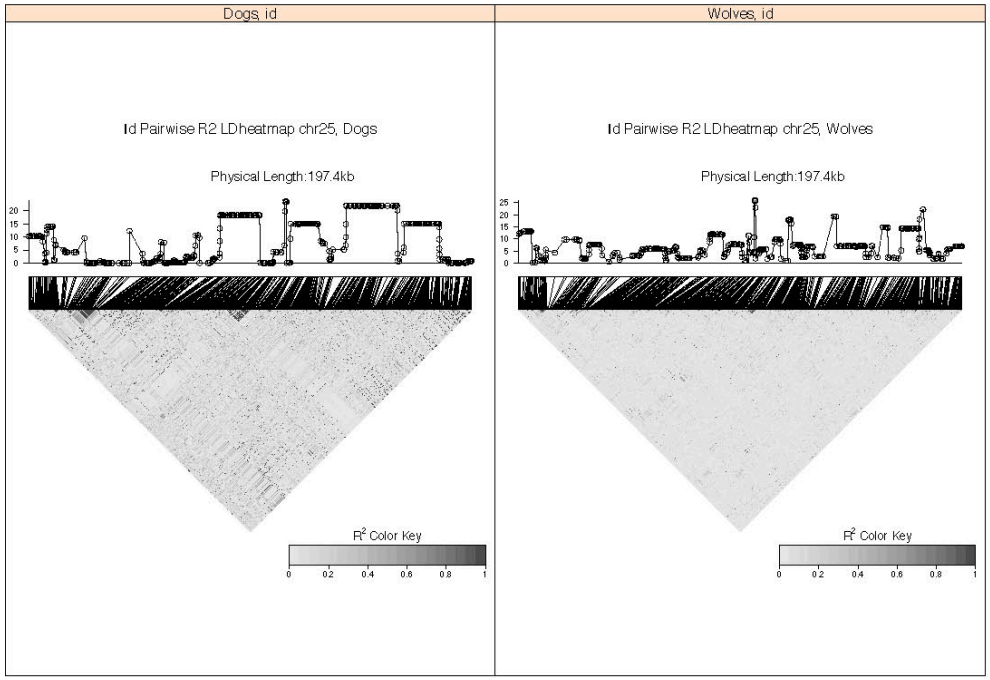
Physical Length:199kb
R2 Color Key
0 0.2 0.4 0.6 0.8 1

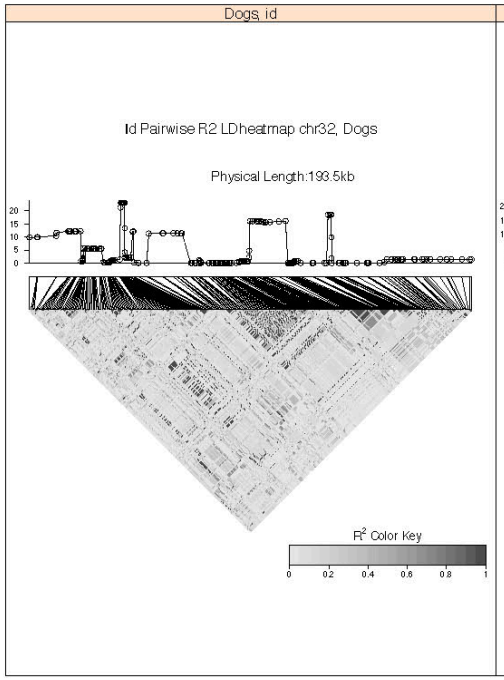
Wolves, id
Id Pairwise R2 LDheatmap chr17, Wolves
Physical Length:199.1kb
R2 Color Key
0 0.2 0.4 0.6 0.8 1

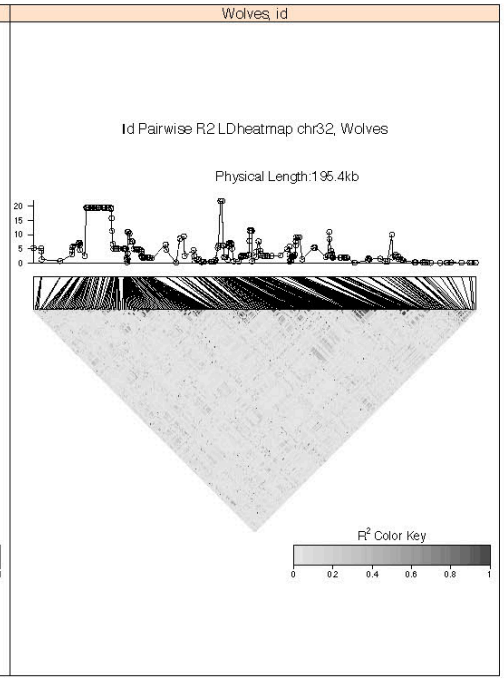

Dogs, id
Id Pairwise R2 LDheatmap chr18b, Dogs
Physical Length:333kb
R2 Color Key
0 0.2 0.4 0.6 0.8 1

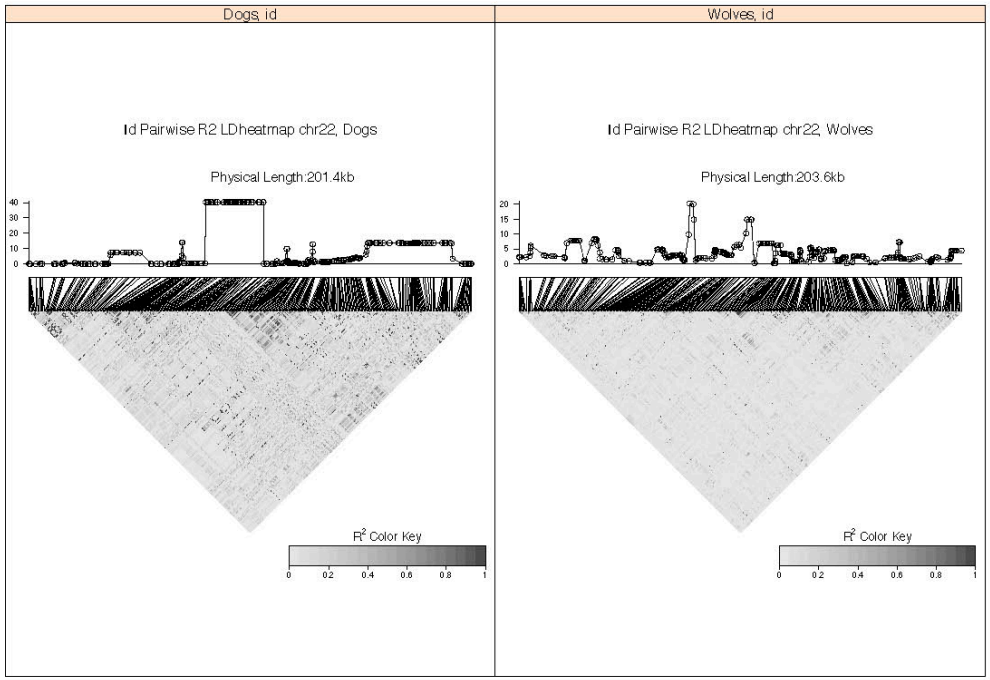

Wolves, id
Id Pairwise R2 LDheatmap chr18b, Wolves
Physical Length:333kb
R2 Color Key
0 0.2 0.4 0.6 0.8 1


Dogs, id
Id Pairwise R2 LDheatmap chr25, Dogs
Physical Length:197.4kb
R² Color Key

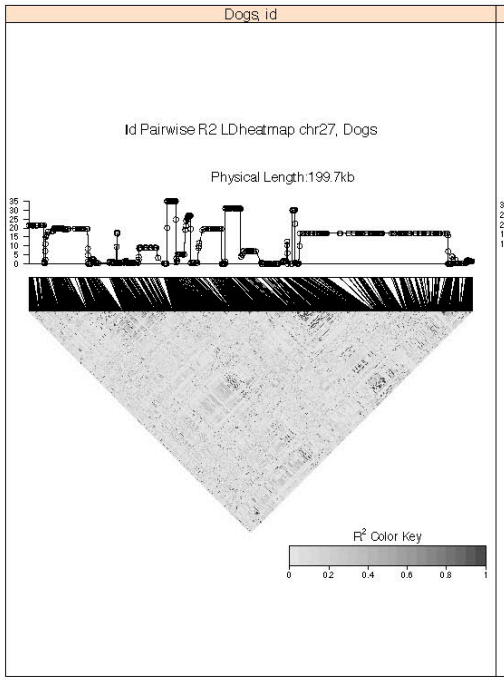
Wolves, id
Id Pairwise R2 LDheatmap chr25, Wolves
Physical Length:197.4kb
R² Color Key

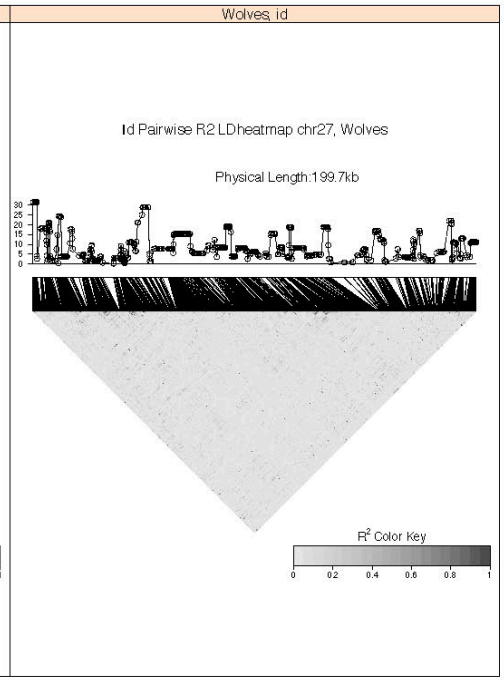

Dogs, id
Id Pairwise R2 LDheatmap chr32, Dogs
Physical Length:193.5kb
R² Color Key

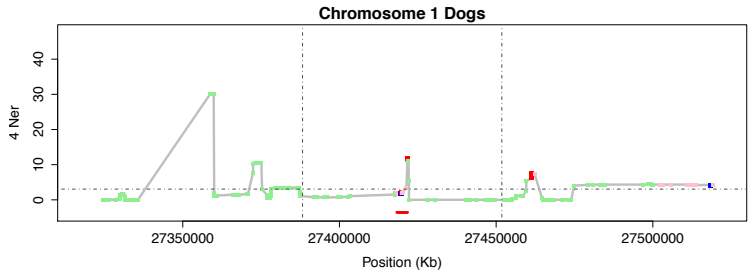

Wolves, id
Id Pairwise R2 LDheatmap chr32, Wolves
Physical Length:195.4kb
R² Color Key

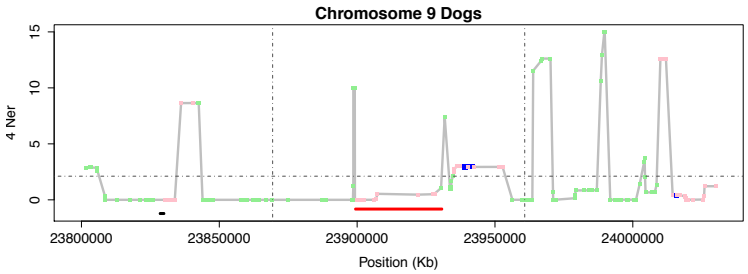

Dogs, id
Id Pairwise R2 LDheatmap chr22, Dogs
Physical Length:201.4kb
R² Color Key

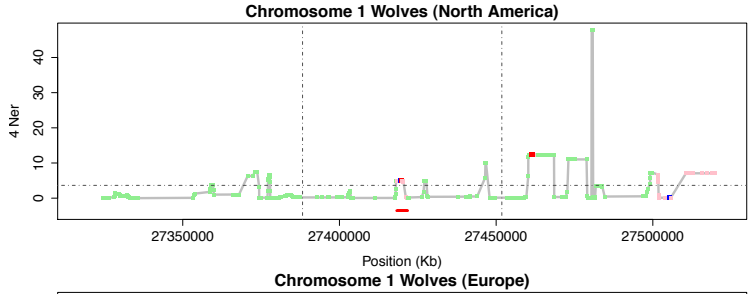
Wolves, id
Id Pairwise R2 LDheatmap chr22, Wolves
Physical Length:203.6kb
R² Color Key

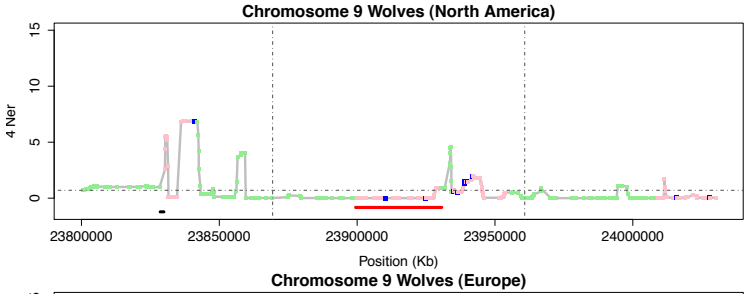

Dogs, id
Id Pairwise R2 LDheatmap chr27, Dogs
Physical Length:199.7kb
R² Color Key

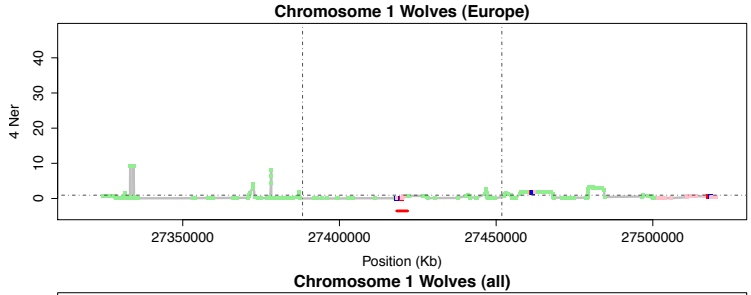

Wolves, id
Id Pairwise R2 LDheatmap chr27, Wolves
Physical Length:199.7kb
R² Color Key

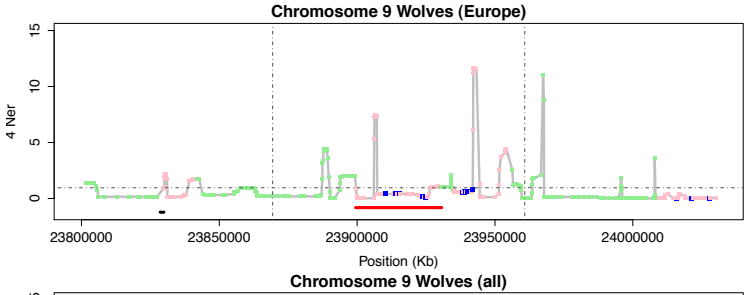

Chromosome 1 Dogs
4 Ner
0
10
20
30
40
27350000
27400000
27450000
27500000
Position (Kb)

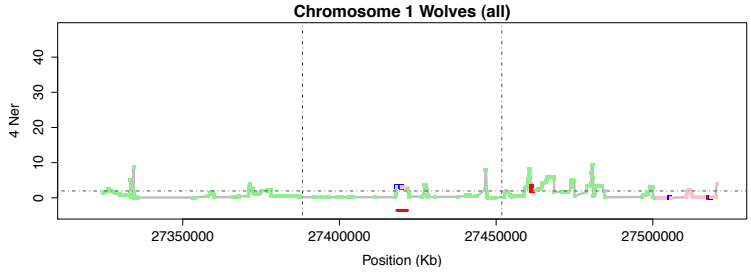

Chromosome 9 Dogs
4 Ner
0
5
10
15
23800000
23850000
23900000
23950000
24000000
Position (Kb)

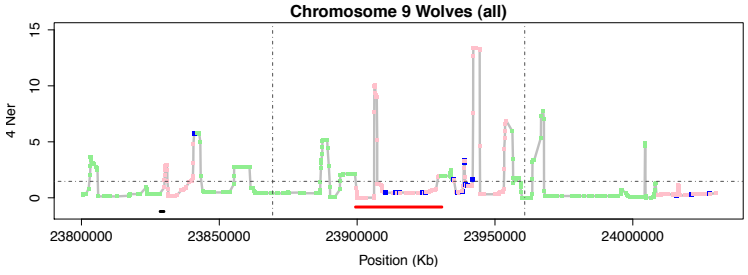

Chromosome 1 Wolves (North America)
4 Ner
0
10
20
30
40
27350000
27400000
27450000
27500000
Position (Kb)

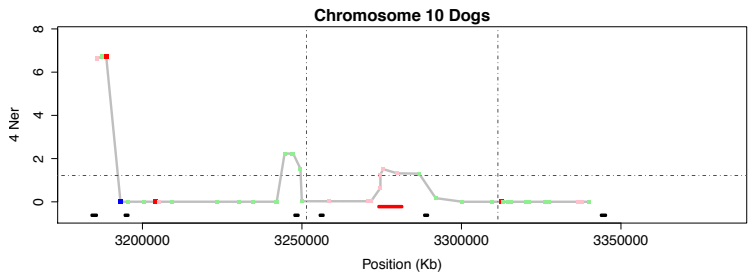

Chromosome 9 Wolves (North America)
4 Ner
0
5
10
15
23800000
23850000
23900000
23950000
24000000
Position (Kb)

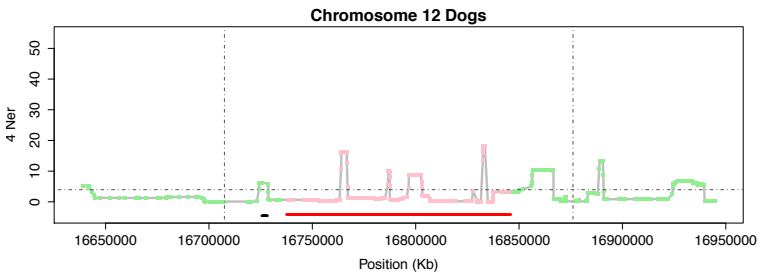

Chromosome 1 Wolves (Europe)
4 Ner
0
10
20
30
40
27350000
27400000
27450000
27500000
Position (Kb)

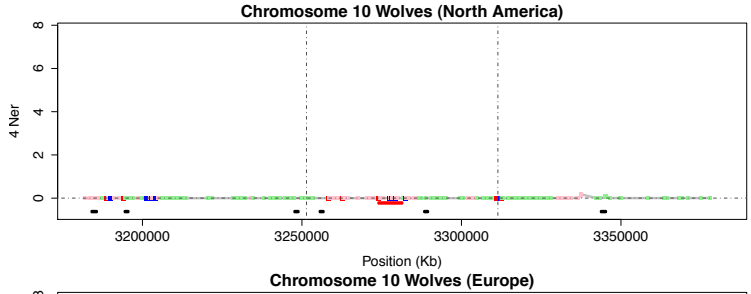

Chromosome 9 Wolves (Europe)
4 Ner
0
5
10
15
23800000
23850000
23900000
23950000
24000000
Position (Kb)

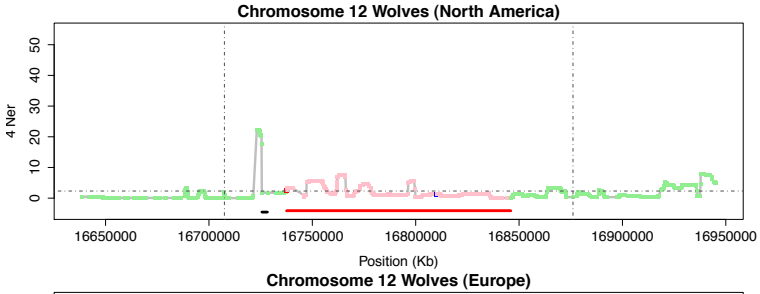

Chromosome 1 Wolves (all)
4 Ner
0
10
20
30
40
27350000
27400000
27450000
27500000
Position (Kb)

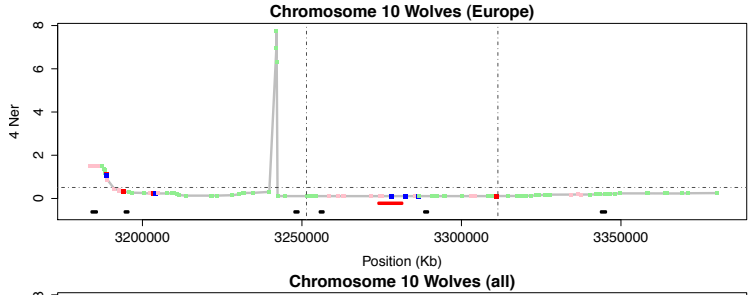

Chromosome 9 Wolves (all)
4 Ner
0
5
10
15
23800000
23850000
23900000
23950000
24000000
Position (Kb)

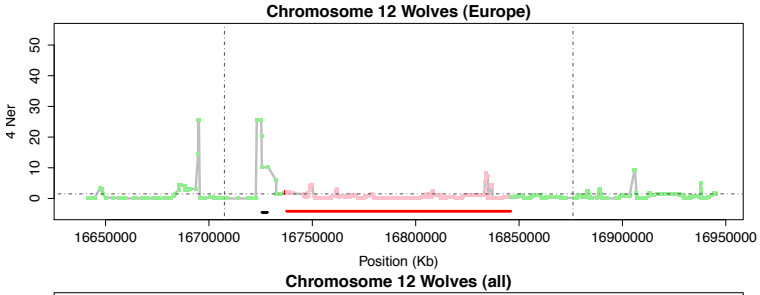

Chromosome 10 Dogs
4 Ner
0
2
4
6
8
3200000
3250000
3300000
3350000
Position (Kb)

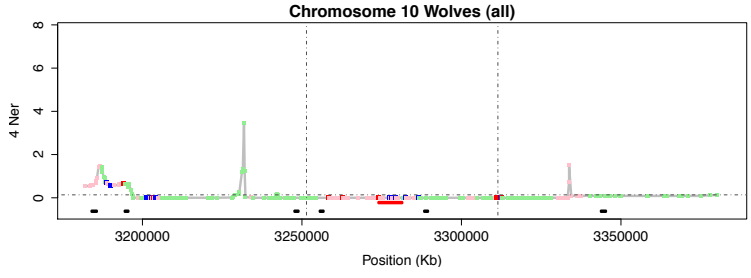

Chromosome 12 Dogs
4 Ner
0
10
20
30
40
50
16650000
16700000
16750000
16800000
16850000
16900000
16950000
Position (Kb)

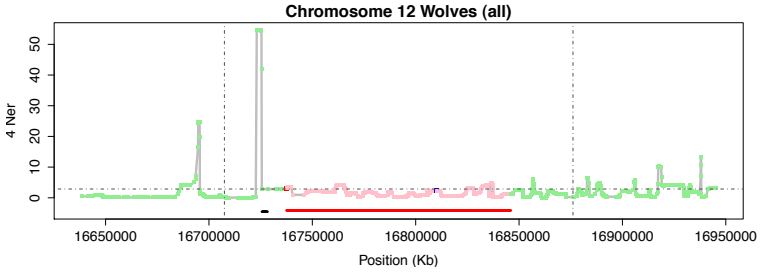

Chromosome 10 Wolves (North America)
4 Ner
0
2
4
6
8
3200000
3250000
3300000
3350000
Position (Kb)

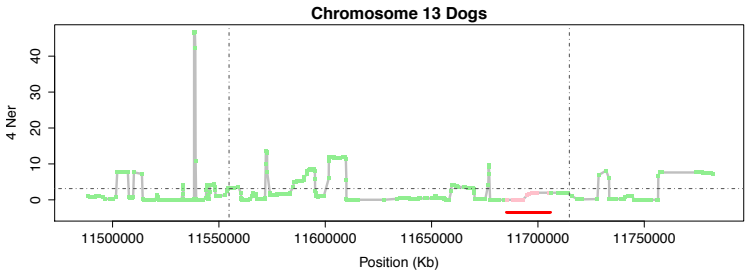

Chromosome 12 Wolves (North America)
4 Ner
0
10
20
30
40
50
16650000
16700000
16750000
16800000
16850000
16900000
16950000
Position (Kb)

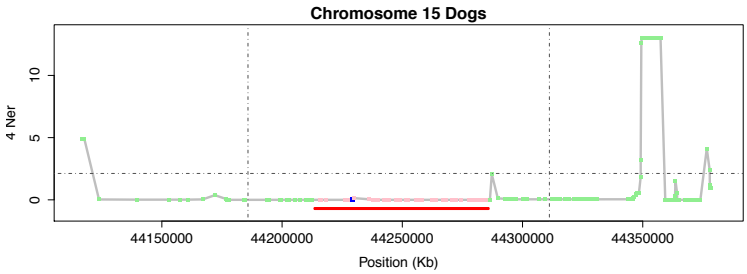

Chromosome 10 Wolves (Europe)
4 Ner
0
2
4
6
8
3200000
3250000
3300000
3350000
Position (Kb)

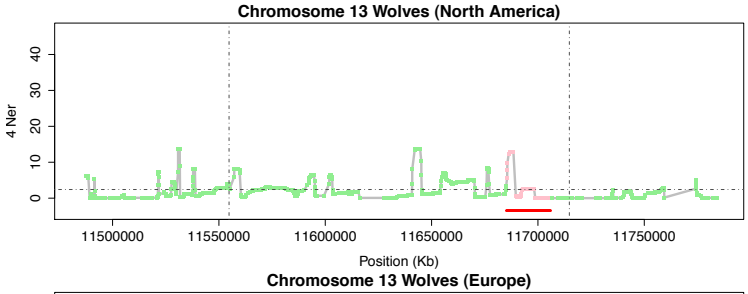

Chromosome 12 Wolves (Europe)
4 Ner
0
10
20
30
40
50
16650000
16700000
16750000
16800000
16850000
16900000
16950000
Position (Kb)

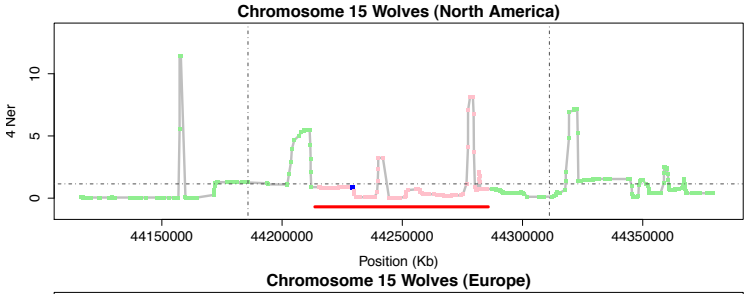

Chromosome 10 Wolves (all)
4 Ner
0
2
4
6
8
3200000
3250000
3300000
3350000
Position (Kb)

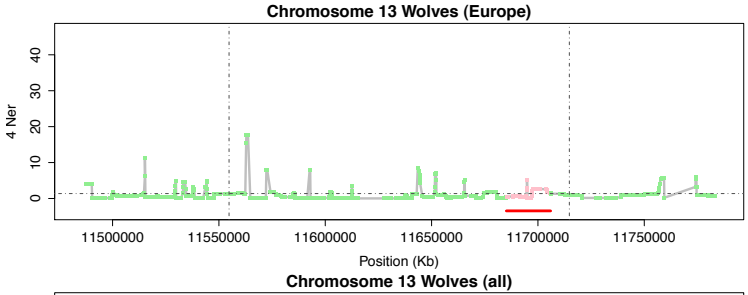

Chromosome 12 Wolves (all)
4 Ner
0
10
20
30
40
50
16650000
16700000
16750000
16800000
16850000
16900000
16950000
Position (Kb)

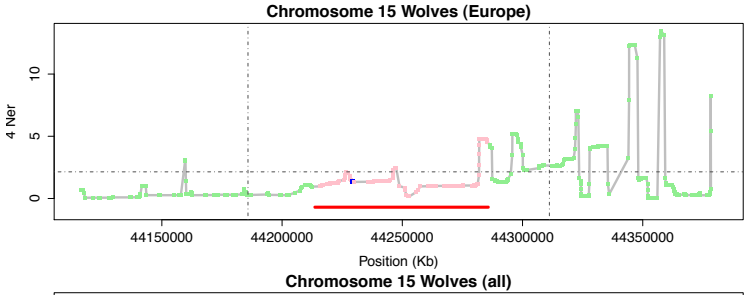

Chromosome 13 Dogs
4 Ner
0
10
20
30
40
11500000
11550000
11600000
11650000
11700000
11750000
Position (Kb)

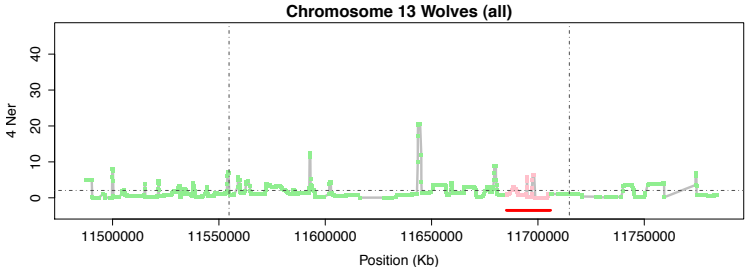

Chromosome 15 Dogs
4 Ner
0
5
10
44150000
44200000
44250000
44300000
44350000
Position (Kb)

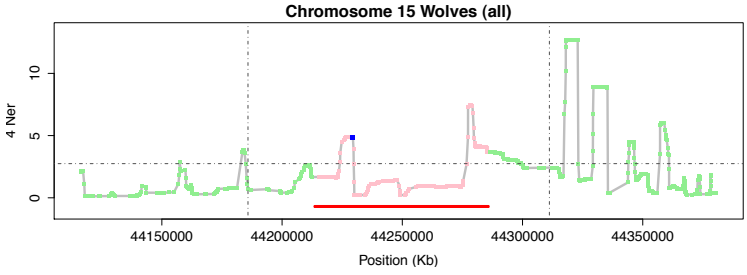

Chromosome 13 Wolves (North America)
4 Ner
0
10
20
30
40
11500000
11550000
11600000
11650000
11700000
11750000
Position (Kb)

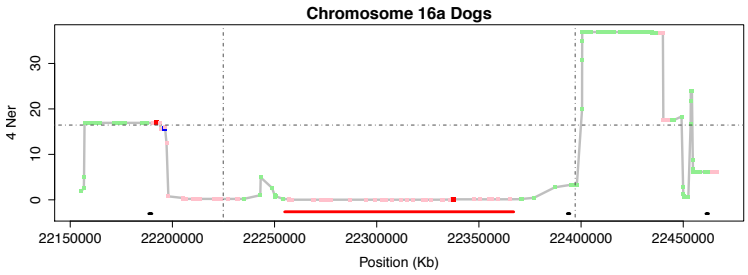

Chromosome 15 Wolves (North America)
4 Ner
0
5
10
44150000
44200000
44250000
44300000
44350000
Position (Kb)

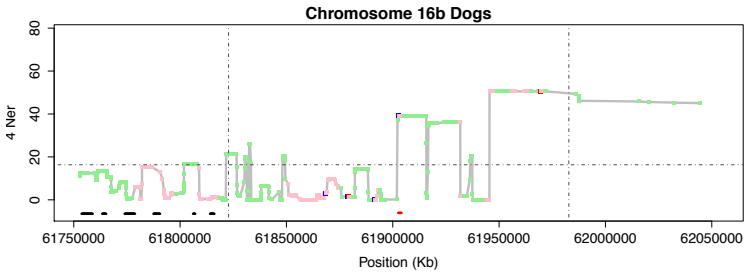

Chromosome 13 Wolves (Europe)
4 Ner
0
10
20
30
40
11500000
11550000
11600000
11650000
11700000
11750000
Position (Kb)

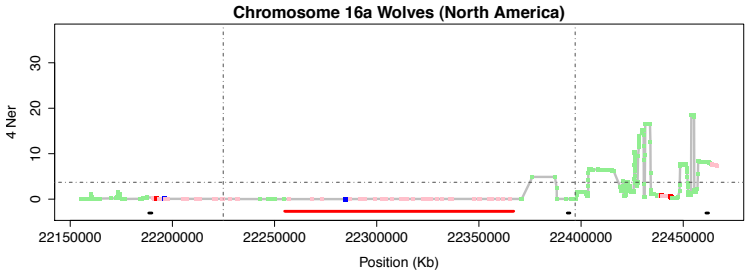

Chromosome 15 Wolves (Europe)
4 Ner
0
5
10
44150000
44200000
44250000
44300000
44350000
Position (Kb)

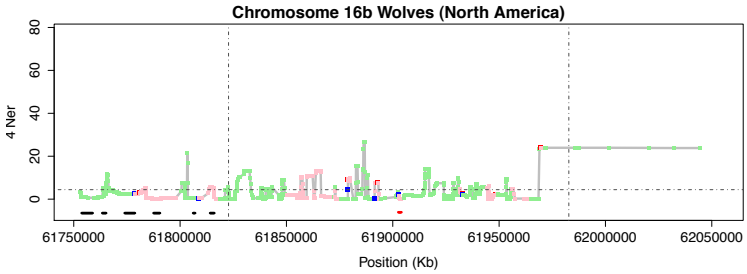

Chromosome 13 Wolves (all)
4 Ner
0
10
20
30
40
11500000
11550000
11600000
11650000
11700000
11750000
Position (Kb)

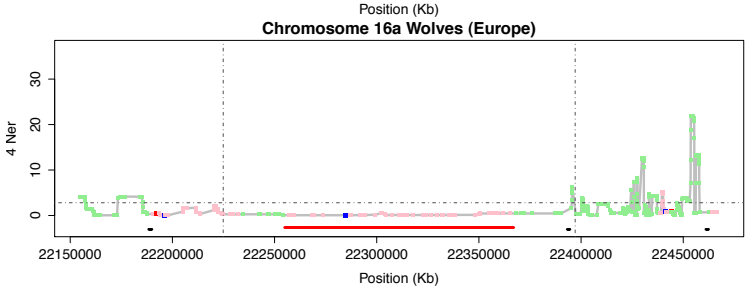

Chromosome 15 Wolves (all)
4 Ner
0
5
10
44150000
44200000
44250000
44300000
44350000
Position (Kb)

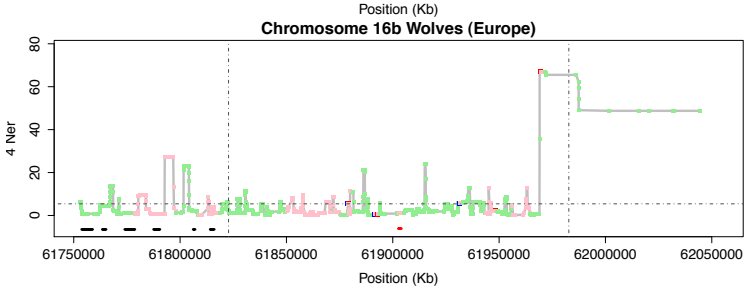

Chromosome 16a Dogs
4 Ner
0
10
20
30
22150000
22200000
22250000
22300000
22350000
22400000
22450000
Position (Kb)

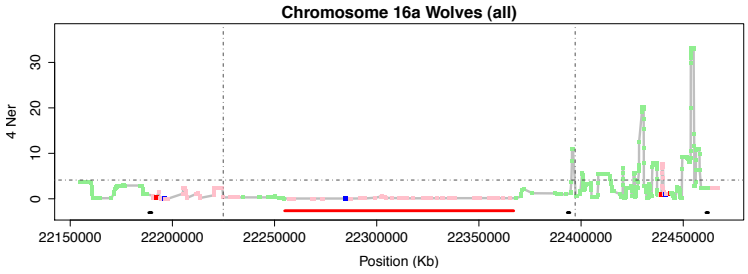

Chromosome 16b Dogs
4 Ner
0
20
40
60
80
61750000
61800000
61850000
61900000
61950000
62000000
62050000
Position (Kb)

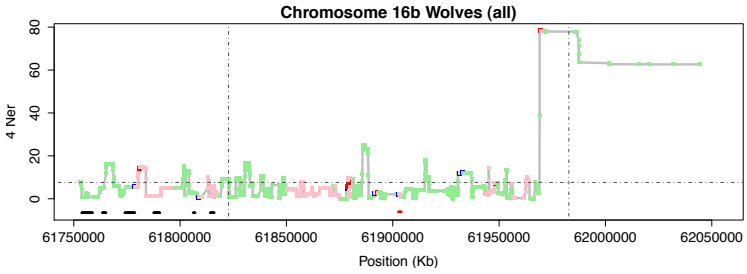

Chromosome 16a Wolves (North America)
4 Ner
0
10
20
30
22150000
22200000
22250000
22300000
22350000
22400000
22450000
Position (Kb)

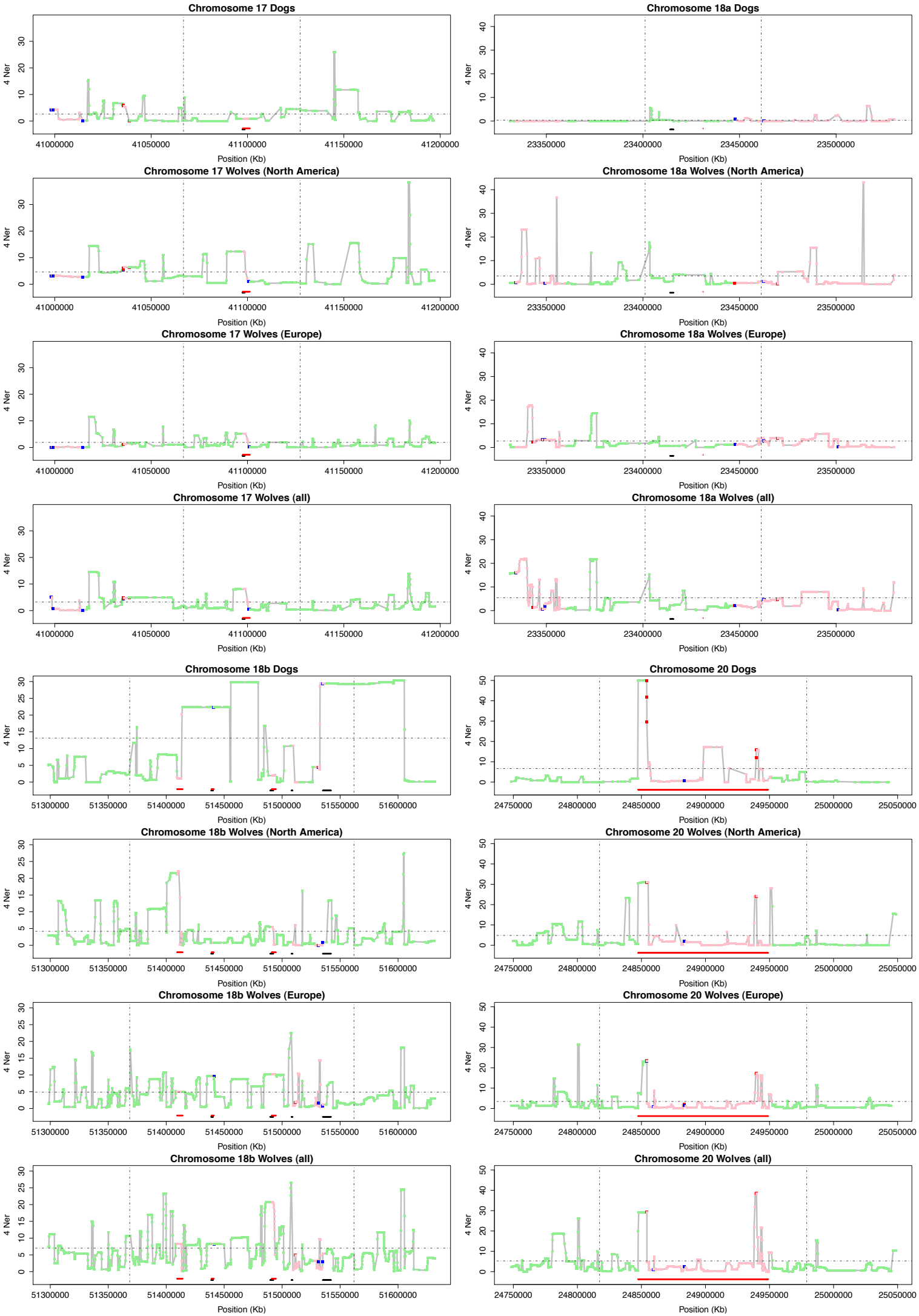

Chromosome 16b Wolves (North America)
4 Ner
0
20
40
60
80
61750000
61800000
61850000
61900000
61950000
62000000
62050000
Position (Kb)

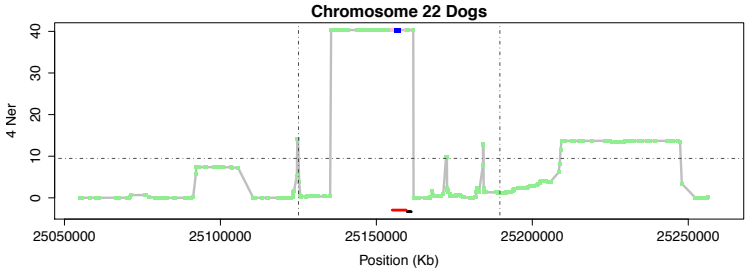

Chromosome 16a Wolves (Europe)
4 Ner
0
10
20
30
22150000
22200000
22250000
22300000
22350000
22400000
22450000
Position (Kb)

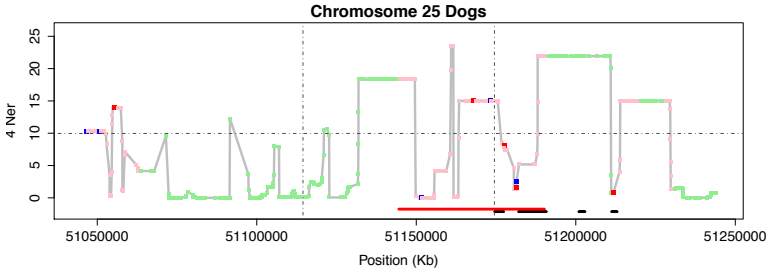

Chromosome 16b Wolves (Europe)
4 Ner
0
20
40
60
80
61750000
61800000
61850000
61900000
61950000
62000000
62050000
Position (Kb)

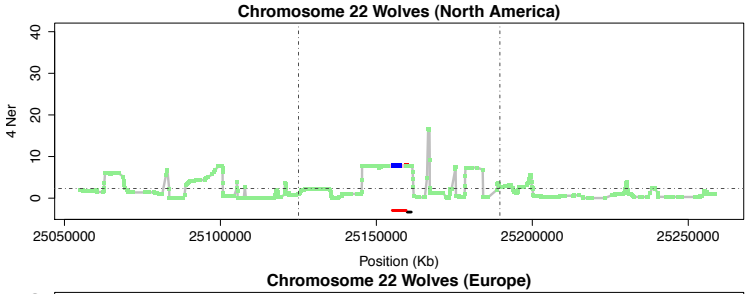

Chromosome 16a Wolves (all)
4 Ner
0
10
20
30
22150000
22200000
22250000
22300000
22350000
22400000
22450000
Position (Kb)

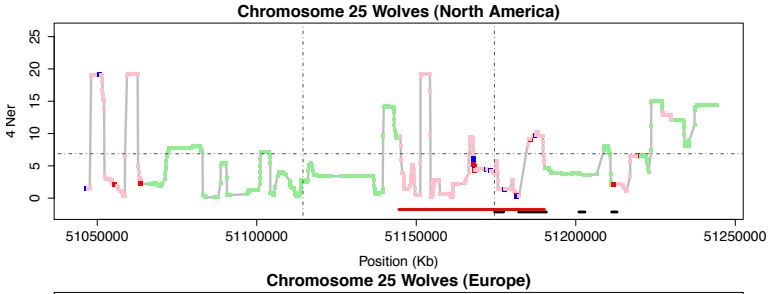

Chromosome 16b Wolves (all)
4 Ner
0
20
40
60
80
61750000
61800000
61850000
61900000
61950000
62000000
62050000
Position (Kb)

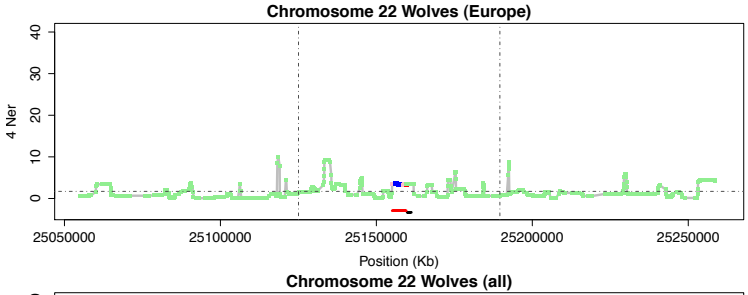

Chromosome 17 Dogs
Chromosome 18a Dogs
Chromosome 17 Wolves (North America)
Chromosome 18a Wolves (North America)
Chromosome 17 Wolves (Europe)
Chromosome 18a Wolves (Europe)
Chromosome 17 Wolves (all)
Chromosome 18a Wolves (all)
Chromosome 18b Dogs
Chromosome 20 Dogs
Chromosome 18b Wolves (North America)
Chromosome 20 Wolves (North America)
Chromosome 18b Wolves (Europe)
Chromosome 20 Wolves (Europe)
Chromosome 18b Wolves (all)
Chromosome 20 Wolves (all)
4 Ner
Position (Kb)

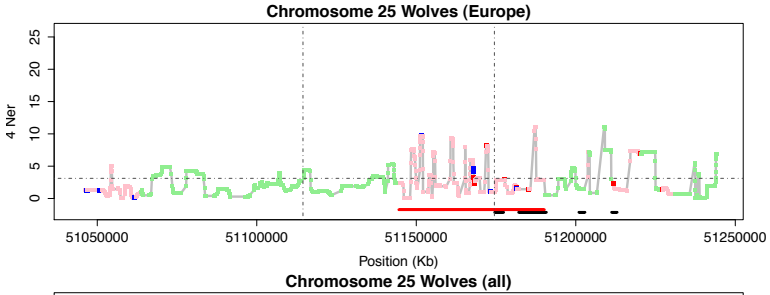

Chromosome 22 Dogs
4 Ner
0
10
20
30
40
25050000
25100000
25150000
25200000
25250000
Position (Kb)

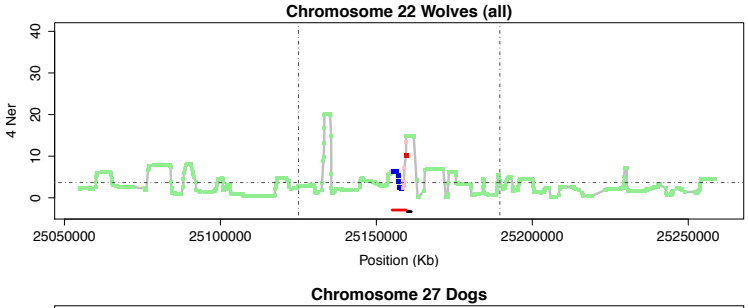

Chromosome 25 Dogs
4 Ner
0
5
10
15
20
25
51050000
51100000
51150000
51200000
51250000
Position (Kb)

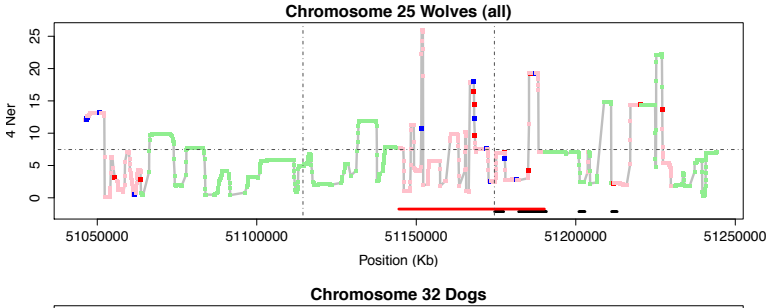

Chromosome 22 Wolves (North America)
4 Ner
0
10
20
30
40
25050000
25100000
25150000
25200000
25250000
Position (Kb)

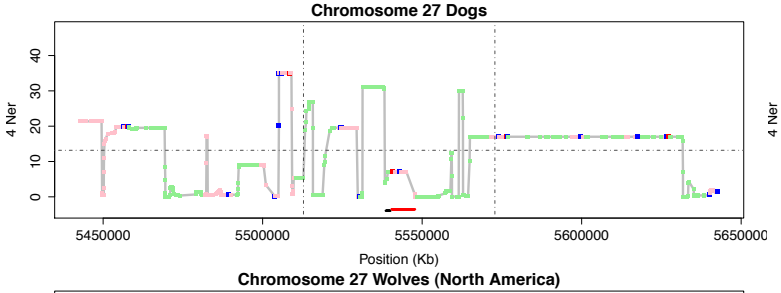

Chromosome 25 Wolves (North America)
4 Ner
0
5
10
15
20
25
51050000
51100000
51150000
51200000
51250000
Position (Kb)

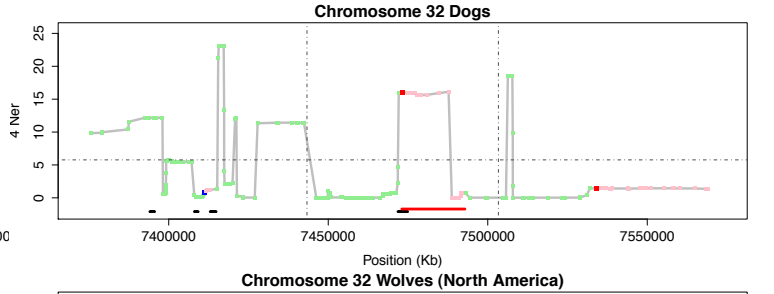

Chromosome 22 Wolves (Europe)
4 Ner
0
10
20
30
40
25050000
25100000
25150000
25200000
25250000
Position (Kb)

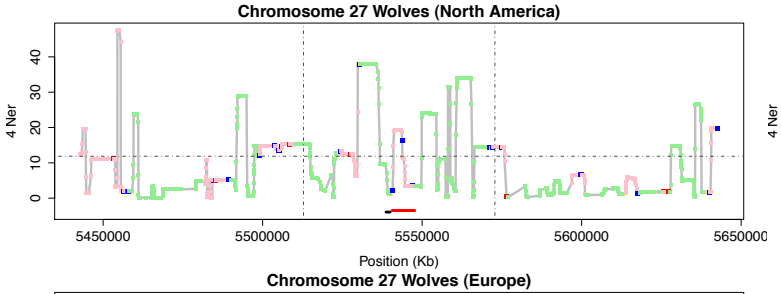

Chromosome 25 Wolves (Europe)
4 Ner
0
5
10
15
20
25
51050000
51100000
51150000
51200000
51250000
Position (Kb)

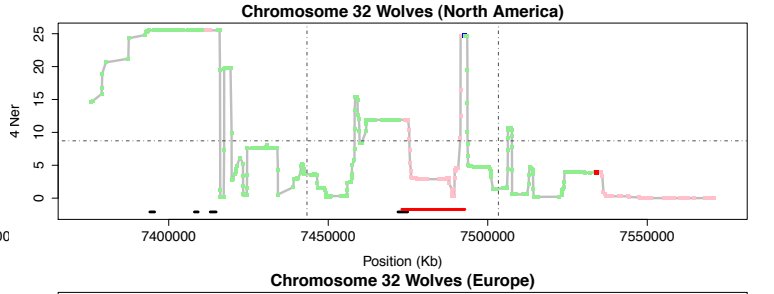

Chromosome 22 Wolves (all)
4 Ner
0
10
20
30
40
25050000
25100000
25150000
25200000
25250000
Position (Kb)

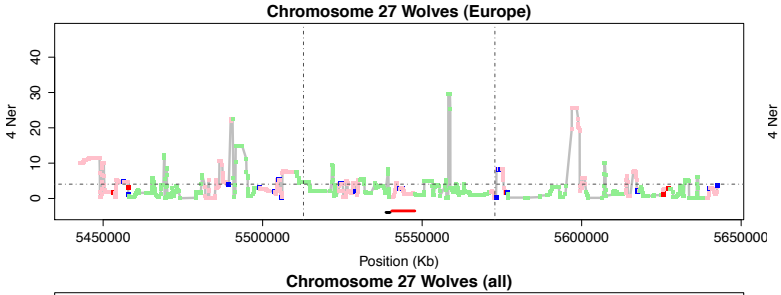

Chromosome 25 Wolves (all)
4 Ner
0
5
10
15
20
25
51050000
51100000
51150000
51200000
51250000
Position (Kb)

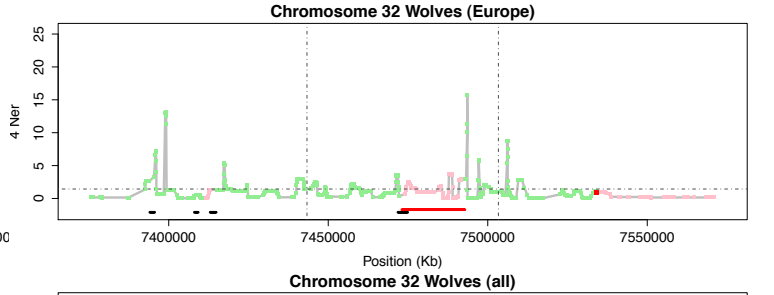

Chromosome 27 Dogs
4 Ner
0
10
20
30
40
5450000
5500000
5550000
5600000
5650000
Position (Kb)

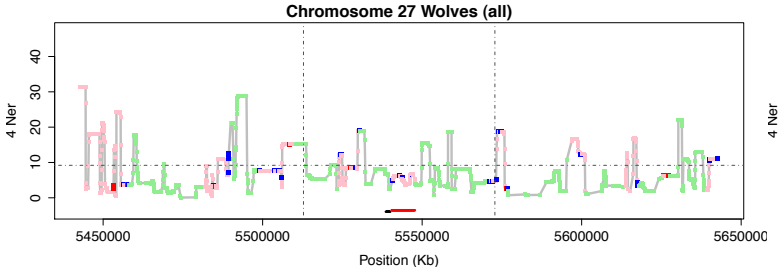

Chromosome 32 Dogs
4 Ner
0
5
10
15
20
25
7400000
7450000
7500000
7550000
Position (Kb)

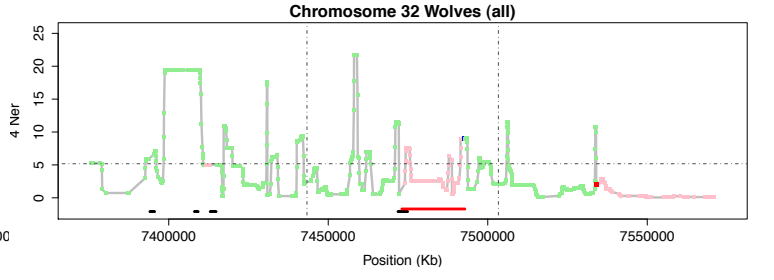

Chromosome 27 Wolves (North America)
4 Ner
0
10
20
30
40
5450000
5500000
5550000
5600000
5650000
Position (Kb)


Chromosome 32 Wolves (North America)
4 Ner
0
5
10
15
20
25
7400000
7450000
7500000
7550000
Position (Kb)


Chromosome 27 Wolves (Europe)
4 Ner
0
10
20
30
40
5450000
5500000
5550000
5600000
5650000
Position (Kb)


Chromosome 32 Wolves (Europe)
4 Ner
0
5
10
15
20
25
7400000
7450000
7500000
7550000
Position (Kb)


Chromosome 27 Wolves (all)
4 Ner
0
10
20
30
40
5450000
5500000
5550000
5600000
5650000
Position (Kb)


Chromosome 32 Wolves (all)
4 Ner
0
5
10
15
20
25
7400000
7450000
7500000
7550000
Position (Kb)