1 **The Japanese wolf is most closely related to modern dogs and its ancestral genome**

2 **has been widely inherited by dogs throughout East Eurasia**

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Abstract

The Japanese wolf (*Canis lupus hodophilax* Temminck, 1839) was a subspecies of the gray wolf that inhabited the Japanese Archipelago and became extinct 100-120 years ago. In this study, we determined the whole genomes of nine Japanese wolves from the 19th- early 20th centuries and 11 Japanese dogs and analyzed them along with both modern and ancient wolves and dogs. Genomic analyses indicate that the Japanese wolf was a unique subspecies of the gray wolf that was genetically distinct from both modern and ancient gray wolves, lacking gene flow with other gray wolves. A Phylogenetic tree that minimizes the effects of introgression shows that Japanese wolves are closest to the dog monophyletic group among the gray wolves. Moreover, Japanese wolves show significant genetic affinities with East Eurasian dogs. We estimated the level of introgression from the ancestor of the Japanese wolves to the ancestor of East Eurasian dogs that had occurred in the transitional period from the Pleistocene to the Holocene, at an early stage after divergence from West Eurasian dog lineages. Because of this introgression, Japanese wolf ancestry has been inherited by many dogs through admixture between East Eurasian dog lineages. As a result of this heredity, up to 5.5% of modern dog genomes throughout East Eurasia are derived from Japanese wolf ancestry.

Introduction

The phylogeny of gray wolves (*Canis lupus*) attracts wide attention from researchers and the public because wolves are the closest relatives to one of the most familiar animal species to humans, i.e., dogs. The extant gray wolves (*Canis lupus*) are divided into three lineages: the North American, Eurasian, and domestic dog lineages, including several now-extinct lineages that inhabited Eurasia during the Pleistocene (Ramos-Madrigal, et al. 2021). Recent phylogenomic analyses of gray wolves have shown that the North American gray wolf diverged at the basal ancestral position, followed by the Eurasian lineage (Fan, et al. 2016; Leathlobhair, et al. 2018). Dogs form a monophyletic clade which is the sister group to the Eurasian lineage of the gray wolf (Freedman, et al. 2014; Fan, et al. 2016; Leathlobhair, et al. 2018). Therefore, the hypothesis that the dog lineages have originated in Eurasia has been widely accepted. But there is still much debate concerning when, where, how many times, and from which population, the ancestor of dogs was domesticated (Leonard, et al. 2002; Savolainen, et al. 2002; Germonpré, et al. 2009; Pang, et al. 2009; Vonholdt, et al. 2010; Larson, et al. 2012; Axelsson, et al. 2013; Thalmann, et al. 2013; Freedman, et al. 2014; Perri 2016; Janssens, et al. 2018; Leathlobhair, et al. 2018; Perri, et al. 2021). Because no extant population of gray wolves has been reported to be more closely related to dogs than the other wolf populations, it is believed that the dog lineage has been domesticated from an extinct population of gray wolves (Larson, et al. 2012; Thalmann, et al. 2013; Freedman, et al. 2014; Skoglund, et al. 2015; Fan, et al. 2016; Frantz, et al. 2016). However, no information is available about this extinct population.

Many regions in Eurasia, including southern East Asia (Savolainen, et al. 2002; Pang, et al. 2009; Wang, et al. 2013; Wang, et al. 2016), Middle East (Vonholdt, et al. 2010), Central Asia

59   (Shannon, et al. 2015), Europe (Thalmann, et al. 2013), and both West and East Eurasia (dual

60   origin) (Frantz, et al. 2016), have been proposed as candidates for the origin of dogs, but the

61   debate on the origin (single, or dual) as well as the timing of domestication still continues.

62   Divergence between the Eurasian gray wolf and dog lineages has been estimated to be 20,000-

63   40,000 years ago (Skoglund, et al. 2015; Botigué, et al. 2017). Based on phylogenomic analyses,

64   dogs were initially reported to be genetically divided into two distinct lineages, i.e., the West and

65   East Eurasian lineages (Freedman, et al. 2014; Shannon, et al. 2015; Frantz, et al. 2016; Botigué,

66   et al. 2017; Leathlobhair, et al. 2018). Subsequent reports suggested an ancient divergence of the

67   Arctic sled dog lineage (Sinding, et al. 2020), which is closely related to the pre-contact American

68   dogs (Leathlobhair, et al. 2018). The phylogenetic relationship between the Arctic sled dog

69   lineage and the West and East Eurasian lineages is conflicting (Larson, et al. 2012; Frantz, et al.

70   2016; Wang, et al. 2016), and these inconsistent topologies can be explained by either a high

71   degree of admixture after the divergence of the three lineages, or by nearly simultaneous

72   divergence (Frantz, et al. 2016; Zhang, et al. 2020). The West Eurasian and East Eurasian lineages

73   diverged 17,000-24,000 years ago (Botigué, et al. 2017), and the Arctic sled dog lineage is

74   estimated to have existed at least 9500 years ago (Sinding, et al. 2020).

75   Studies have suggested that wolf populations in Europe (Thalmann, et al. 2013), the Middle East

76   (Vonholdt, et al. 2010), Central Asia (Shannon, et al. 2015), Siberia (Sinding, et al. 2020; Ramos-

77   Madrigal, et al. 2021), and East Asia (Savolainen, et al. 2002; Wang, et al. 2016) have undergone

78   introgression or bidirectional gene flow (Freedman, et al. 2014) with dogs. However, genomic

79   introgression from gray wolves to dogs has been considered to be limited due to the presence of

80   Eurasian wolves that do not show genetic affinity to any dog breed (Bergström, et al. 2020).

81   The Japanese wolf (*Canis lupus hodophilax* Temminck, 1839) was a subspecies of the gray wolf

82   that inhabited Honshu, Shikoku, and Kyushu Islands in the Japanese Archipelago and became

83   extinct 100-120 years ago (Ishiguro, et al. 2009). Molecular phylogenetic analysis of the

84   mitochondrial genome suggests that the Japanese wolf diverged at the basal position of the extant

85   gray wolf clade (Matsumura, et al. 2014; Matsumura, et al. 2020). Recent genome analysis of a

86   "Honshu wolf" (one of the common names for the Japanese wolf) from the collection of the

87   British Museum suggests that this individual is closely related to a lineage of Siberian wolves that

88   existed in the Late Pleistocene and shows significant gene flow with Japanese dogs (Niemann, et

89   al. 2021).

90   In this study, the genomes of nine Japanese wolves, including the type specimens, and 11

91   Japanese dogs were newly determined and analyzed. The analyses showed that 1) the Japanese

92   wolf was a unique subspecies of the gray wolf that is genetically distinct from both extant and

93   ancient gray wolves known to date, 2) the Japanese wolf is most closely related to a monophyletic

94   group of dogs, and 3) Japanese wolf ancestry has introgressed into the ancestor of East Eurasian

95   dogs at an early stage of their history after diverging from the West Eurasian lineages, and the

96   genome derived from Japanese wolf ancestry has been inherited by many modern dogs, even in

97   the West Eurasian lineages, through their historical admixture with East Eurasian lineages.

98

99   **Results**

100   **Relationships between Japanese wolves and other dogs and gray wolves**

101   In this study, original genomic DNA sequences of nine Japanese wolves (22-282 Gb) and 12

102   Japanese dogs (70-127 Gb) were determined (Table S1, Fig. S1). For the present study, we treated

103   nine individuals with Japanese wolf type mitochondrial DNA haplotypes (Matsumura, et al. 2020)

104   as the Japanese wolf. In addition, we used sequencing data of modern gray wolves with depth of

105   coverage >20x in the database, ancient canids with relatively high coverage, and outgroup species

106   from the database (Table S2). All sequence data were mapped to the reference genome sequence

107   (CanFam3.1). After haplotype calling and gvcf file merging, single nucleotide polymorphisms

108   (SNPs) were genotyped. To examine the genetic relationship among the individuals used in this

109   study, a principal component analysis (PCA) was performed using individuals with high coverage

110   (Fig. 1A). In the PCA, Japanese wolves formed a distinct cluster, suggesting that Japanese wolves

111   were genetically separated from dogs, gray wolves, and any of the outgroup species. The gray

112   wolves were clustered into two groups, i.e., a North American/Arctic group and a Eurasian group

113   along the PC1 axis. Dogs show an East-West cline along the PC2 axis. Dingoes and New Guinea

114   singing dogs (NGSD) were the closest to Japanese wolves among dogs along the PC2 axis,

115   followed by a cluster of Japanese dogs (Fig. 1A). Using the same data set we also generated an

116   ADMIXTURE result with the lowest CV error at K=4 (Fig. 1B). In this analysis, Japanese wolves

117   also formed clusters with higher K such as K=5 or K=6, indicating that their genetic composition

118   was unique compared with that of the other dogs and gray wolves (Fig. 1B and S2).

119   Next, we added the three Japanese wolf individuals with low coverage [Leiden b and c, (Jentink

120   1887), and a "Honshu wolf" (Niemann, et al. 2021), see Table S2] into the analysis. PCA showed

121   that Leiden b and Honshu wolf were very close to the Japanese wolf cluster, while Leiden c was

122   placed at an intermediate position between dogs and Japanese wolves (Fig. S3). ADMIXTURE

123   analysis showed that Leiden b and Honshu wolf exhibit the same ancestry pattern as the other

124   Japanese wolf individuals, while Leiden c seemed to be admixed with other dogs (Fig. S4). We

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125   used Patterson's $f4$ statistic (Patterson, et al. 2012) to identify dog individuals with high genetic

126   affinity to Leiden c to see which dog population was the source of the introgression. The dog that

127   showed the highest affinity to Leiden c was the Japanese dog Shiba (Fig. S5A), and Leiden c

128   contained 39% of the Shiba's genome (Fig. S5B). In contrast, Leiden b showed no affinity with

129   dogs (Fig. S5C). These results indicate that Leiden b and Honshu wolf are included in the group

130   of Japanese wolves with a unique genetic composition, while Leiden c is a hybrid individual

131   between Japanese wolves and dogs. Subsequently, we removed Leiden b, c and Honshu wolf

132   individuals from further analyses due to their low coverage.

133   A previous study (Niemann, et al. 2021) suggested that Honshu wolf has a close relationship

134   with a lineage of Siberian wolves that existed in the Late Pleistocene and was likely to be admixed

135   with dogs. Therefore, we added the Pleistocene wolves to our dataset which are comprised of the

136   modern gray wolves and dogs and performed PCA (Fig. S6). Pleistocene wolves were placed

137   closely related to Eurasian wolves, while Japanese wolves formed a distinct cluster. Our

138   ADMIXTURE analysis (Fig. S4) suggests that Honshu wolf does not contain more DNA

139   components of dogs than the other Japanese wolf individuals. The differences between the results

140   of Niemann et al. 2020 and this study could be caused by differences in the number of Japanese

141   wolves used in the analyses and/or in the coverage of DNA sequencing.

142

143   **Phylogenetic position of the Japanese wolves**

144   To determine the phylogenetic position of the Japanese wolves, a phylogenetic tree was

145   constructed using the maximum likelihood (ML) method (Fig, 2A). Among gray wolves, North

146   American/Arctic individuals branched off first at the basal position of the tree, followed by

147   European/Middle Eastern and East Asian gray wolves (also see Fig. S7). Dogs formed a

148   monophyletic clade including East Eurasian, West Eurasian, and sled dog lineages (Fig. 2A, S7),

149   as shown in previous studies (Freedman, et al. 2014; Shannon, et al. 2015; Frantz, et al. 2016;

150   Botigué, et al. 2017; Leathlobhair, et al. 2018; Sinding, et al. 2020). Japanese wolves formed a

151   monophyletic clade that was a sister group to the monophyletic clade of dogs (Fig. 2A, S7). The

152   sister group relationship between Japanese wolves and dogs was also supported by a tree inferred

153   by SVDQuartets and a Neighbor-Joining tree based on identity-by-state (IBS) distance (Figs. S8,

154   S9). Analysis using outgroup-*f3* statistics (Patterson, et al. 2012) also showed that the Japanese

155   wolf was the most closely related to dogs among wolves (Fig. 2B). When we further divide the

156   dogs into subpopulations, outgroup-*f3* statistics showed different results between dingo/NGSD

157   and African dogs; dingo/NGSD was related most closely to Japanese wolf while African dog is

158   related most closely to the Middle Eastern gray wolves (Fig. S10A and S10B). The different

159   genetic affinities of dog populations to the Japanese wolf may have resulted from introgression

160   between African dogs and Middle Eastern gray wolves (Vonholdt, et al. 2010; Bergström, et al.

161   2020).

162   Since the tree topology in phylogenetic analyses could be affected by introgression between taxa,

163   a phylogenetic tree using taxa showing minimal introgression effects is expected to be the most

164   accurate representation of population branching. Therefore, in order to obtain such a tree, we

165   examined introgression between Japanese wolves and other dogs and wolves. We compared the

166   genetic affinity of each dog with the Japanese wolves using *f3* and *f4* statistics, and found that

167   dogs of the East Eurasian lineage (Fig. S7), in particular, dingoes, NGSDs, and Japanese dogs,

168   showed significant affinity with the Japanese wolves (Z score > 3) (Fig. 2C, S11). In contrast,

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169   dogs of the west Eurasian lineage, in particular dogs from Africa, showed low affinity to Japanese

170   wolves (Fig. 2C, S11). *f4* statistics showed no affinity between any of the gray wolf populations

171   and Japanese wolves (Fig. 2D). Possibilities of gene flow between gray wolves except for the

172   Japanese wolf and dogs were also examined using *f4* statistics. Gray wolves in the Middle East

173   showed strong affinity with dogs (Fig. S12), consistent with previous reports (Vonholdt, et al.

174   2010; Bergström, et al. 2020). Based on these results, we reperformed phylogenetic analysis to

175   confirm the relationship between Japanese wolves and dogs. To minimize the effect of

176   introgression between wolves and dogs, we included African dogs as the sole representatives of

177   dogs, and excluded gray wolves from the Middle East. Even in the phylogenetic tree obtained

178   from this analysis, the Japanese wolf still formed a sister clade with African dogs (Fig. S13). Thus,

179   we concluded that the most closely related wolves to the dog lineage are the Japanese wolves.

180

181   **The genome of the Japanese wolf ancestor in the dog genome**

182   Japanese wolves showed strong affinity with many East Eurasian dogs (*f3*, *f4* statistics) (Figs. 2C,

183   S11), which may be caused by the introgression of dog genomes into Japanese wolves (Japanese

184   wolf or its ancestors, hereafter simply referred to as the "the Japanese wolf genome") or vice versa.

185   A recent report suggested widespread gene flow from dogs to gray wolves, but little gene flow

186   from gray wolves to dogs, based on the existence of gray wolves that have no affinity with dogs

187   (Bergström, et al. 2020). We investigated the direction of gene flow between Japanese wolves and

188   East Eurasian dogs using the *f4*-ratio (Patterson, et al. 2012). We found that the degree of genome

189   introgression from the Japanese wolf lineage to dogs was the highest in dingoes and NGSDs

190   (5.5%) followed by Japanese dogs (3-4%), as well as in dogs of other East Eurasian lineages (Fig.

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191   3A). In contrast, genomic introgression from dogs to the Japanese wolf genome was not supported

192   (Fig. S14). We further analyzed the possibility of a small proportion of genomic introgression

193   from dogs to the Japanese wolf genome by the $f4$ statistics. If the dog genome is introgressed into

194   the Japanese wolf genome, the genetic affinities between the Japanese wolf and dogs would be

195   different between individuals. Indeed, the degree of affinity of Japanese wolves with

196   dongo/NGSD varied among individuals (Fig. S15) and Jw258, Jw269, and Jw271 showed

197   significant affinity to Japanese dogs (Fig. S16). This difference in affinity suggests that the

198   Japanese wolf genome contains a small proportion of the dog genome that is undetectable by $f4$-

199   ratio.

200   The degree of genomic introgression from Japanese wolves to dogs was higher in East Eurasian

201   than in West Eurasian dogs. It also varied among the dogs of East Eurasia. This variation may

202   have been caused by multiple introgression events between the ancestors of Japanese wolves and

203   dogs in different regions, or by a single introgression followed by diffusion of the Japanese wolf

204   genome into various dog populations. To determine which hypothesis is more likely, we first

205   examined the degree of gene flow among dogs in different regions. African dogs and dingo/NGSD

206   represent opposite edges of the dog cluster in the PCA (Fig. 1A), and show the lowest and highest

207   affinities with Japanese wolves, respectively (Fig. 2C). Among dogs, African dogs show the

208   lowest affinity with dingo/NGSD, while dingo/NGSDs show the lowest affinity with African dogs

209   (Fig. S17, S18). Dingoes are estimated to have been introduced to Australia between 4600 and

210   18300 years ago based on their mitochondrial DNA (Oskarsson, et al. 2012), with archaeological

211   evidence supporting the arrival of at least 3500 years ago (Milham and Thompson 1976). It is

212   considered that the dingoes have been isolated in Australia since then (Larson, et al. 2012; Fan,

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213   et al. 2016). African dogs are estimated to have migrated to Africa 14,000 years ago (Liu, et al.

214   2018) and have been isolated since then (Larson, et al. 2012; Fan, et al. 2016). The African Dog

215   and Dingo/NGSD are included in the West Eurasian and East Eurasian lineages, respectively (Fig.

216   S7). Therefore, they are likely to be the oldest dogs of their respective lineages. The $f4$ statistics

217   biplot showed that dogs showing higher affinity with dingo/NGSD show lower affinity with

218   African dogs while dogs showing higher affinity with African dogs show lower affinity with

219   Dingo/NGSD (Fig. S19). This negative correlation suggests most of dog populations were formed

220   through extensive past mixing between East and West Eurasian lineages represented by

221   dingo/NGSD and African dogs, respectively. Indeed, several dogs in South and East Asia are

222   genomically characterized as dingo/NGSD admixed with African dogs by negative values of $f3$

223   statistics (Patterson, et al. 2012) (Fig. S20).

224   Next, we examined the degree of introgression between dogs from different regions and

225   Japanese wolves. TreeMix analysis indicates an introgression from the ancestor of Japanese

226   wolves into the common ancestor of dingo/NGSD and Japanese dogs (Fig. 3B). The $f3$ biplot of

227   affinities with dingo/NGSD and with Japanese wolves shows a positive correlation among dogs,

228   indicating that the Japanese wolf genome has become widespread through the admixture between

229   West and East Eurasian dog lineages and persists in the modern genomes of the East Eurasian

230   lineage (Fig. 3C). Therefore, it is likely that the genome of the Japanese wolf ancestor was

231   introgressed into an ancestral lineage of the East Eurasian lineage after the split of West and East

232   Eurasian lineages. Subsequently, the East Eurasian lineage containing the Japanese wolf ancestry

233   admixed with the West Eurasian lineage, resulting in differences in affinities with the Japanese

234   wolves. The Southeast Asian dogs (Fig. 3C green) have a relatively higher affinity with

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235   Dingo/NGSD compared to their affinity with Japanese wolves, suggesting gene flow between the

236   Southeast Asian dogs and dogs that carried no Japanese wolf genome after the first admixture

237   event. Similarly, Japanese dogs (Fig. 3C blue) have a relatively higher affinity with Japanese

238   wolves compared to their affinity with Dingo/NGSD, suggesting subsequent gene flow between

239   Japanese dogs and Japanese wolves. Hence, it is likely that the difference in the degree of genomic

240   introgression from Japanese wolves to dogs was caused by a single introgression followed by

241   diffusion of the Japanese wolf genome into various dog populations.

242

243   **Genomic regions derived from Japanese wolf that contribute to the traits of Japanese dogs**

244   The results of *f4*-ratio analysis showed that Akita, Shiba, and Kishu dogs contain 36-45% West

245   Eurasian dog genomes (Table S3). It is expected that the genomic regions containing genes

246   responsible for traits of the Japanese dogs are highly differentiated from those of West Eurasian

247   dogs. If such regions overlap with the Japanese wolf-derived genomic regions (3-4%) in the

248   modern Japanese dog genome, it could be that Japanese wolf-derived genes are responsible for

249   the characteristic traits of the Japanese dog. To investigate such regions, we examined genomic

250   regions derived from Japanese wolves using a f_{dM} sliding window and regions differentiated

251   between Japanese dogs and West Eurasian dogs using an F_{ST} sliding window, and extracted

252   overlapping regions. Six regions were found, which included four genes and the upstream or

253   downstream regions of four genes (Fig. 3D, Fig. S21, Table S5). These regions may be candidates

254   of genomic regions derived from Japanese wolf that contribute to the traits of Japanese dogs.

255

256   **Discussion**

257   The Japanese wolves are likely to have been isolated in the Japanese archipelago until their

258   extinction only 100 years ago. This study reveals that they form a monophyletic group with no

259   evidence for gene flow with other Eurasian gray wolves.

260   One notable aspect of Japanese wolves is their phylogenetic position. In Eurasia, our

261   phylogenetic analysis showed that the European lineage of the gray wolves diverged at the basal

262   position, followed by the Middle East and East Asian lineages. In the East Asian lineage, the

263   monophyletic group of Japanese wolves and the dog lineage form a sister-group relationship. The

264   order in which Eurasian wolf lineages diverged is from west to east in geographical order on the

265   Eurasian continent. Considering these phylogenetic and geographic relationships, it is most likely

266   that it was in East Asia that the divergence between the Japanese wolf and the dog lineages has

267   occurred. In other words, the extinct population of the gray wolf from which dogs are suspected

268   to have been domesticated (Larson, et al. 2012; Thalmann, et al. 2013; Freedman, et al. 2014;

269   Skoglund, et al. 2015; Fan, et al. 2016; Frantz, et al. 2016) is closely related to the ancestor of the

270   Japanese wolf and is likely to inhabit East Asia. This hypothesis does not directly imply that the

271   origin of dog domestication was in East Asia, nor does it directly imply that the earliest dogs

272   descended from the ancestor of Japanese wolves. Although the domestication process would have

273   been initiated with the animals' association with humans (Larson, et al. 2012; Perri 2016; Janssens,

274   et al. 2018), our phylogenetic analyses provide no evidence for when dog lineages began to

275   associate with humans. Further archaeological evidence in the studies of ancient "proto-dog"

276   populations are required to clarify the beginnings of the dog-human relationship.

277   This study suggests ancient genomic introgression from the Japanese wolf to dogs, most likely

278   to the ancestor of the East Eurasian lineage. The divergence between the dog lineage and the

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279   Eurasian gray wolves has been estimated to be 20,000-40,000 years ago (Skoglund, et al. 2015;

280   Botigué, et al. 2017). Dogs have been reported to have split into West Eurasian, East Eurasian,

281   and sled dog lineages in their early divergence (Freedman, et al. 2014; Shannon, et al. 2015;

282   Frantz, et al. 2016; Botigué, et al. 2017; Leathlobhair, et al. 2018). Because a 9500-year-old sled

283   dog (Sinding, et al. 2020) already contained the same proportion of the Japanese wolf genome as

284   the modern sled dog (2%: Table S4), the genomic introgression of the ancestor of the Japanese

285   wolf to the East Eurasian lineage of dogs must have occurred before the establishment of the sled

286   dog lineage at least 9500 years ago during the transitional period from the Pleistocene to the

287   Holocene and shortly after the divergence of the East and West Eurasian dog lineages. The

288   genome of NGSD was estimated to contain the Japanese wolf genome (5.5%). It is estimated that

289   the NGSD lineage already existed by 10,900 years ago (Bergström, et al. 2020), which also

290   supports the hypothesis that the introgression from the ancestor of Japanese wolves into dogs had

291   occurred in the Pleistocene. The ancient dog genome data from two European individuals (4,800

292   and 7,000 years ago) already contained about 1.6% of the Japanese wolf genome (Table S4). Since

293   the gene flow from the Southeast Asian dog ancestry to the ancestor of these two ancient European

294   dogs has been reported (Botigué, et al. 2017), the genome of the Japanese wolf may have been

295   transmitted to European dogs via the Southeast Asian dog ancestry more than 7,000 years ago.

296   Although the Japanese wolf has only ever been found living in the Japanese archipelago, it is

297   unlikely that the introgression between the ancestor of the Japanese wolf and dogs of the East

298   Eurasian lineage occurred in the Japanese archipelago. An ancient sled dog (Sinding, et al. 2020)

299   excavated at the same time as the excavation of the oldest dog in Japan (9600 years ago)

300   (Shigehara and Hongo 2000) already possessed the Japanese wolf genome, suggesting that

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301   introgression between the ancestor of Japanese wolves and dogs of the East Eurasian lineage had

302   occurred before dogs were brought to the Japanese archipelago. Therefore, the introgression

303   between the ancestral Japanese wolf and the East Eurasian lineage of dogs is most likely to have

304   occurred somewhere in East Asia.

305   The dogs with a high proportion of the Japanese wolf genome are the dingo/NGSD (5.5%) and

306   the Japanese dogs (3-4%). The high proportion of the dingo/NGSD is inferred to be due to their

307   isolation in the islands of Southeast Asia and Australia, where they have escaped admixture with

308   the West Eurasian dog lineage. NGSD is estimated to be an admixture of two lineages (Bergström,

309   et al. 2020), and thus one of the admixed lineages may have possessed a higher proportion (~10%)

310   of the Japanese wolf genome than NGSD. In contrast, Japanese dogs contain about 36-45% of the

311   West Eurasian genome (Table S3), even though a high proportion of the Japanese wolf genome

312   persists in their genomes. After the first introgression with the East Eurasian dog ancestry, the

313   Japanese wolf genome may have introgressed into the Japanese dog genomes in the Japanese

314   archipelago. This hypothesis is supported by the ratio of affinity with Japanese wolves and with

315   dingoes/NGSDs, which tends to be higher in Japanese dogs (Fig. 3C). In addition, the Japanese

316   dogs have the highest affinity to the Japanese wolf among all dogs (Fig. S16), suggesting that the

317   Japanese wolf genome was recently introgressed into the Japanese dog genome.

318   Although only a small proportion of the Japanese wolf genome persists in modern dog genomes,

319   the Japanese wolf genome might have an effect on dog characteristic traits. We searched for

320   genomic regions in Japanese dogs that derived from the Japanese wolf, and that were highly

321   differentiated between Japanese dogs and West Eurasian dogs. Six regions were found with these

322   criteria (Fig. 3D, Fig. S21, Table S5). Despite the gene flow from Eurasian dogs, these regions

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323   were differentiated between the genomes of Japanese and West Eurasian dogs. Therefore, these

324   regions are expected to have been selected in the Japanese dogs and are the candidates of genomic

325   regions potentially responsible for phenotypic characteristics of Japanese dogs. Further analysis

326   of the regions selected in East Eurasian dogs and a genome-wide association study for East

327   Eurasian dog traits should reveal the effects of the Japanese wolf genome on dog traits.

328   In this study, we demonstrated that the Japanese wolf is a sister group with the monophyletic

329   clade of dogs. Our original results support the hypothesis that the modern dog lineage was

330   domesticated from an extinct population of gray wolves (Larson, et al. 2012; Thalmann, et al.

331   2013; Freedman, et al. 2014; Skoglund, et al. 2015; Fan, et al. 2016; Frantz, et al. 2016), and the

332   Japanese wolf is the closest to this now-extinct gray wolf population. In addition, we estimated

333   the levels of introgression from the ancestor of Japanese wolves to the ancestor of East Eurasian

334   dogs. Accordingly, the Japanese wolf genome is expected to be involved in the early stages of

335   dog domestication. Further analysis of the genome of the Japanese wolf and ancient dog genomes,

336   in particular from East Eurasia, will continue to shed light on the origins of dog domestication.

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338

339   **Materials and methods**

340   **Samples, DNA extraction, and sequencing**

341   Japanese Wolf and Japanese dog DNAs were extracted and used in our previous studies

342   (Matsumura, et al. 2014; Matsumura, et al. 2020). The sample locations are listed in Table S1.

343   DNAs of two individuals of Shiba (Shiba_shiro and Shiba_kuro) were extracted from blood

344   samples using a DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions.

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345 The NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Bio Labs, Ipswich, MA,

346 USA) was used to construct libraries from genomic DNA following the manufacturer's

347 instructions. Paired-end (2 × 150 bp) sequencing was performed on the Illumina HiSeq X or

348 NovaSeq 6000 platforms.

349 For Leiden b and Leiden c, the genome capture was performed using the SeqCap EZ

350 Hybridization and Wash Kit (Roche, Basel, Switzerland), SeqCap EZ Accessory Kit v2 (Roche),

351 SeqCap HE-Oligo Kit (Roche), and SeqCap EZ Pure Capture Bead Kit (Roche) following the

352 manufacturer's instructions for SeqCap EZ Library SR (Roche), with minor modifications.

353 Briefly, Biotin-labeled genomic DNA fragments from Shiba were used as hybridization probes,

354 instead of the SeqCap EZ library (Roche). Leiden b and Leiden c libraries were mixed with 135

355 ng of Biotin-labeled genomic DNA fragments and were hybridized at 47°C for 72 h. Other

356 procedures were performed in accordance with the manufacturer's instructions. Total mapped

357 reads are listed in Table S1.

358

359 **Extraction of SNPs and vcf file preparation**

360 Sequence reads from the genomic DNA libraries of nine Japanese wolves, eleven Japanese dogs

361 (Table S1) as well as 88 wolves/dogs and six outgroup species from the database (Table S2) were

362 trimmed to remove adaptor sequences and mapped to the dog reference genome (CanFam3.1)

363 using CLC Genomics Workbench (https://www.qiagenbioinformatics.com/). Reads showing high

364 similarity (> 90% in > 90% of read length) were mapped to the reference genome sequences and

365 reads mapped to more than one position were removed ("ignore" option for reads mapped to

366 multiple positions). The mapping data was exported in bam file format and sorted and indexed

17

367 using samtools (Li, et al. 2009). The duplicated reads in bam files were marked by the

368 MarkDuplicates algorithm implemented in GATK v4.2 (https://gatk.broadinstitute.org/hc/en-us).

369 We performed genotype calling on all individuals analyzed in this study using the

370 HaplotypeCaller algorithm in GATK v4.2. Genotypes of all individuals were output as gvcf

371 format (-ERC GVCF option) and filtered by the VariantFiltration algorithm in GATK v4.2 with

372 default parameters. All gvcf files were combined into a single gvcf format file by the

373 CombineGVCFs algorithm in GATK v4.2. The combined file was genotyped by the

374 GenotypeGVCFs algorithm and filtered by Filtervcf in GATK v4.2 with parameters; --filter-

375 expression "QD < 2.0" --filter-name "QD2" --filter-expression "QUAL < 30.0" --filter-name

376 "QUAL30" --filter-expression "FS > 200.0" --filter-name "FS200" --filter-expression "SOR >

377 10.0" -filter-name "SOR10" --filter-expression "ReadPosRankSum < -20.0" --filter-name

378 "ReadPosRankSum-20".

379   To maximize the number of SNPs for analyses without missingness, we prepared vcf files for

380 each analysis using vcftools (Danecek, et al. 2011). When including all individuals except for

381 ancient samples and Leiden b, Leiden c, and Honshu wolf (see the sample combination in Table

382 S2), we removed all indels, singleton, and doubleton sites to eliminate PCR and sequencing errors

383 that may have occurred in one individual, and extracted bi-allelic sites with coverage equal to or

384 more than three in all individuals and with GQ values equal to or more than eight in all individuals.

385 Mutations due to DNA damage at both ends of fragments were less than 1% in Japanese wolves

386 (Fig. S1), therefore we can infer that mutations by DNA damage in the sequences of Japanese

387 wolves were removed by this filtration. For analyses with ancient samples, sites were filtered

388 using the same conditions as with modern samples. In addition, we used only transversion sites

389 for the analyses of ancient samples. For the PCA and ADMIXTURE analyses with Liden b and

390 Liden c, sites with missingness greater than 3% and minor allele frequency (MAF) < 0.04 were

391 excluded. Sites were filtered using the same conditions as with modern samples, and only

392 transversion sites were used.

393

394 **Phylogenetic analysis**

395 The SNPs in a vcf file including dogs, wolves and Japanese wolves (see Table S2) were converted

396 to PHYLIP format. 10 kb sequences from the 5' end of the PHYLIP format file were extracted

397 and a model for Maximum Likelihood method was selected using MEGA ver. X (Kumar, et al.

398 2018). A phylogenetic tree was constructed using the Maximum Likelihood (ML) method using

399 PhyML ver. 3.2 (Guindon, et al. 2010) with a model selection option "-m GTR" and with 100

400 bootstrap replications. ML trees were constructed using all individuals (Fig. S7: 489,524 SNPs),

401 selected individuals (Fig. 2A: 1,971,890 SNPs), and outgroup species (Table S2), wolves,

402 Japanese wolves, and African dogs (Fig. S13: 2,065,200 SNPs).

403 The same vcf file used in the ML method (Fig. S7: 489,524 SNPs) was converted to NEXUS

404 format. A phylogenetic tree was constructed by the svdq algorithm (Chifman and Kubatko 2014)

405 in PAUP* ver. 4a (Wilgenbusch and Swofford 2003) with 100 bootstrap replications (Fig. S8).

406 We used PLINK ver. 1.9 (Purcell, et al. 2007) with an option "—distance 1-ibs" to calculate an

407 Identity By State (IBS) distance matrix using 1,992,260 SNPs. A neighbour joining tree was

408 constructed from the IBS distance matrix using MEGA ver. X (Kumar, et al. 2018)(Fig. S9).

409

410 **Principal component analysis and ADMIXTURE**

411   We performed a principal component analysis (PCA) using PLINK ver. 1.9 (Purcell, et al. 2007)

412   with an option "--indep-pairwise 50 10 0.1" to explore the affinity among gray wolves, Japanese

413   wolves, and dogs (Figure 1A). We also performed PCA with type specimens of Japanese wolf

414   (Fig. S3), and with ancient canids (Fig. S6).

415   ADMIXTURE ver. 1.3 (Alexander and Lange 2011) was run on the dataset of modern samples

416   (Fig. 1B and Fig. S2) and modern specimens with type specimens of Japanese wolf (Fig. S4)

417   assuming 2 to 8 clusters (K=2-8).

418

419   *f3*, *f4* **statistics, and *f4*-ratio**

420   *f*3, *f4* statistics, and *f4*-ratio implemented in ADMIXTOOLS ver. 7.0.1 (Patterson, et al. 2012)

421   were used to evaluate the shared genetic drift among gray wolves, Japanese wolves, and dogs.

422   We used the same SNPs data set used for the phylogenetic analysis for all modern samples

423   (489,524 SNPs). For Liden b and Leiden c analyses, vcf files were prepared to maximize the

424   number of SNPs (Table S2).

425   Outgroup *f3* statistics were calculated to explore shared genetic drift between all dogs and each

426   of the wolves (Fig. 2B), African dogs and each of the wolves (Fig. S10A), dingo/NGSD dogs and

427   each of the wolves (Fig. S10B), Japanese wolf and each of the dogs (Fig. 2C), African dogs and

428   each of the other dogs (Fig. S17A), and dingo/NGSD dogs and each of the other dogs (Fig. S17B).

429   *f3* statistics were calculated to test the genomic mixture of African and dingo/NGSD dogs in all

430   dogs (Fig. S20).

431   *f4* statistics were calculated to explore shared genetic drift between each of the dogs and Leiden

432   c (Fig. S5A: 38,254 SNPs), each of the dogs and Leiden b (Fig. S5C: 83,259 SNPs), each of the

433   wolves and Japanese wolves (Fig. 2D), each of the dogs and Japanese wolves (Fig. S11), each of

434   the dogs and each of the wolves (Fig. S12), dingo/NGSD and Japanese wolves (Fig. S15), each

435   of the dogs and Japanese wolves (Fig. S16), NGSD1 or Basenji and each of the dogs (Fig. S18),

436   and dingoes and each of the other dogs, and African dogs and each of the other dogs (Fig. S19)

437

438   **TreeMix**

439   To examine the admixture events, we used TreeMix (Pickrell and Pritchard 2012) to build a tree

440   with admixture edges. We used major groups of gray wolves and dogs as follows; Gray Wolves

441   (North America: n = 2), Gray Wolves (Canada/Arctic: n = 8), Gray Wolves (East Asia: n =5),

442   Gray Wolves (West Eurasia: n = 7), Japanese Wolves (Japan: n = 7), Dogs (Central Asian: n = 4),

443   Dogs (Europe: n = 11), Dogs (Africa: n = 10), Dogs (sled dogs: n = 4), Dogs (Vietnamese

444   Indigenous: n = 5), Ding/NGSD (Oceania: n = 7), Japanese Dogs (Japan: n = 11), and Dogs

445   (Korea: n = 6). The SNPs were pruned based on linkage disequilibrium (LD-pruning) by using

446   plink with an option "--indep-pairwise 50 10 0.12. As a result of LD-pruning, 150,502 SNPs were

447   used for TreeMix.

448

449   **Assessing DNA damage patterns**

450   We used mapDamage ver. 2.2.0 (Ginolhac, et al. 2011) to assess DNA damage patterns in the

451   Japanese wolf samples sequenced in this study. Mapped reads from the Japanese wolf samples

452   showed slightly increased proportion (equal to or less than 1%) of C to T and G to A substitutions

453   at the 5' and 3' read ends, respectively (Fig. S1).

454

455   **Calculation of maximum contamination rate**

456   We used substitutions in mitochondria DNA specific to Japanese wolf to assess the contamination

457   rate of the other animal DNA in Japanese wolf DNA. Fifteen fixed substitutions unique to

458   Japanese wolf were selected using an alignment of mitochondria DNA sequences including gray

459   wolves, Japanese wolves, and dogs used in previous studies (Matsumura, et al. 2014; Matsumura,

460   et al. 2020). The lowest coverage at fifteen sites was 48 (highest was 28,324) in the mapping

461   result to the mitochondria genome in CanFam3.1. We calculated the average mapping ratio in

462   fifteen sites. The ratio of the reads mapped to the fifteen sites without substitutions specific to

463   Japanese wolf were assumed as the maximum contamination rate (Table S6), because the

464   mitochondria DNA like sequences are found in the nuclear genome.

465

466   **Detection of genomic regions that are derived from Japanese wolf and may have contributed**

467   **to Japanese dog characteristics**

468   The genomic regions containing genes that form the characteristics of the Japanese dog are

469   expected to be largely differentiated between the Japanese dog and the West Eurasian dog. To

470   detect such regions, we examined sequences differentiated between Japanese dogs and West

471   Eurasian dogs by using F_{ST} sliding window analysis. Using SNPs of Japanese dogs and African

472   dogs (Table S2) in a vcf file, F_{ST} values for all sites were computed by vcftools v0.1.16 (Danecek,

473   et al. 2011). The average F_{ST} values in windows of 50 SNPs with a 25 SNPs slide across

474   chromosomes were calculated by RStudio version (1.4.1106) (RStudio Team 2020. RStudio:

475   Integrated Development for R. RStudio, PBC, Boston, MA). The Japanese wolf-derived genomic

476   regions were calculated by f_{dM} values in windows of 50 SNPs with a 25 SNPs slide across

22

477   chromosomes using Dsuite (Malinsky, et al. 2021). Then, we extracted regions of overlap between

478   high F_{ST} regions (top 1%) and the Japanese wolf-derived genomic regions (top 1% of f_{dM} values).

479

480

481   **Availability of data**

482   The nucleotide sequences were deposited in the DDBJ Sequenced Read Archive.

483

487

488   **Acknowledgments**

489

490   **Author contributions**

491   JG: data analyses and manuscript writing; NA: next generation sequencing and vcf file

492   preparation of modern samples; XX: assessing DNA damage patterns; YM: genomic DNA

493   extraction of Shiba individuals and next generation sequencing; SM: preparation of an alignment

494   of mitochondrial DNA sequences and manuscript editing; HT: archeological information for

495   discussion and manuscript editing; NI preparation of genomic DNA of the Japanese wolves and

496   Japanese dogs and manuscript editing; YT: research concept, research plan, next generation

497   sequencing, vcf file preparation, data analyses, and manuscript writing.

498

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621

622   **Figure legends**

623   Figure 1 Relationships between Japanese wolves and other canids

624   (A) Principal Components Analysis (PC1 versus PC2) of 100 samples based on 2,065,200 SNPs

625   (see Table S2 for sample information). Colored circle, square, and triangle correspond to the

27

626   names of dogs or wolves in the panel. (B) ADMIXTURE results based on SNP data for K = 4

627   (see Table S2 for sample information).

628

629   Figure 2 Phylogenetic relationships and genetic affinity between Japanese wolves and other

630   canids. (A) Maximum likelihood tree based on 1,971,890 SNPs. Node labels shown in blue

631   squares indicate bootstrap values out of 100. (B) Shared genetic drift between dogs and gray

632   wolves measured by outgroup $f3$ statistics. Each of all dogs and Japanese wolves were used as

633   populations. Each $f3$ statistical value is plotted in order of highest to lowest value from the top,

634   and the names of the wolves are shown on the right side of the panel. Error bars represent standard

635   errors. (C) Shared genetic drift between Japanese wolf and all dogs measured by outgroup $f3$

636   statistics. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each

637    $f3$ statistical value is plotted in order of highest to lowest value from the top, and the names of the

638   dogs are shown on the left side of the panel. Error bars represent standard errors. (D) $f4$ statistics

639   testing the relationships between the Japanese wolf and all other wolves compared with NGSD1

640   (left panel) and Basenji (right panel). Each Z score is plotted in order of highest to lowest value

641   from the top, and the names of wolves are shown on the left and right sides of the left and right

642   panels, respectively. Gray lines show the Z score -3 and 3.

643

644   Figure 3 Admixture between Japanese wolves and the other canids.

645   (A) $f4$-ratio test to estimate proportion of genome introgression from the Japanese wolf to

646   dogs. Each $f4$-ratio α value is plotted in order of highest to lowest value from the top, and the

647   names of the dogs are shown on the right side of the panel. Error bars represent standard errors.

648   Z score above 3 is colored in blue. (B) TreeMix admixture graph built using LD-pruned data

649   (150,502 sites) on a dataset consisting of 88 dogs/wolves merged into 13 groups according to their

650   phylogenetic relationships. (C) $f3$ statistics testing whether dogs share more alleles with

651   dingo/NGSD (x-axis) or Japanese wolf (y-axis). Dots show the $f3$ statistics, and horizontal and

652   vertical error bars represent standard errors for tests with the African dogs (x-axis) and

653   dingo/NGSD (y-axis), respectively. Each of the Japanese wolves and dingo/NGSD individuals

654   were used as populations. (D) Sliding window analyses of the F_{ST} values (y-axis: upper panel)

655   and f_{dM} (y-axis: lower panel) in windows of 50 SNPs using a 25 SNPs slide across chromosome

656   (x-axis). Dashed lines show the 99th percentiles. Blue arrow indicates overlapping regions above

657   99th percentiles between upper and lower panels. The gene name above the arrow is the gene in
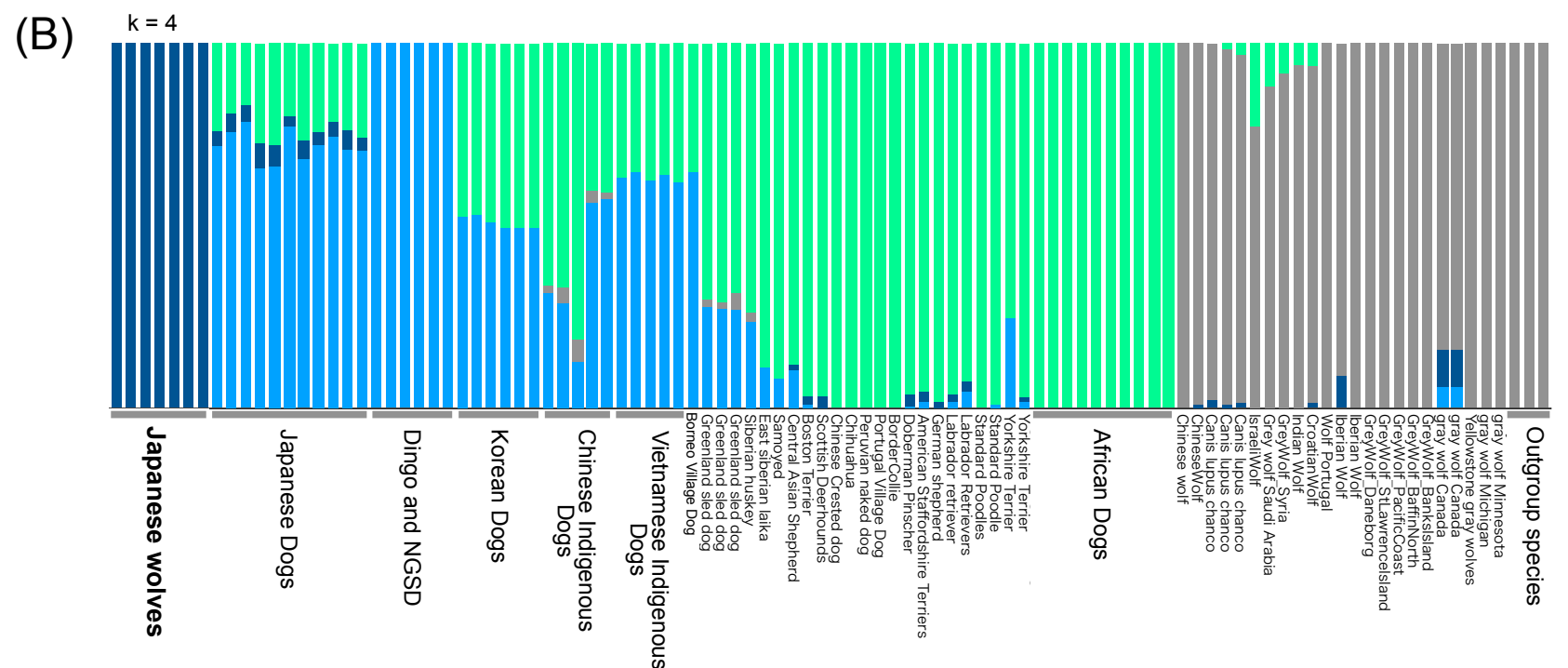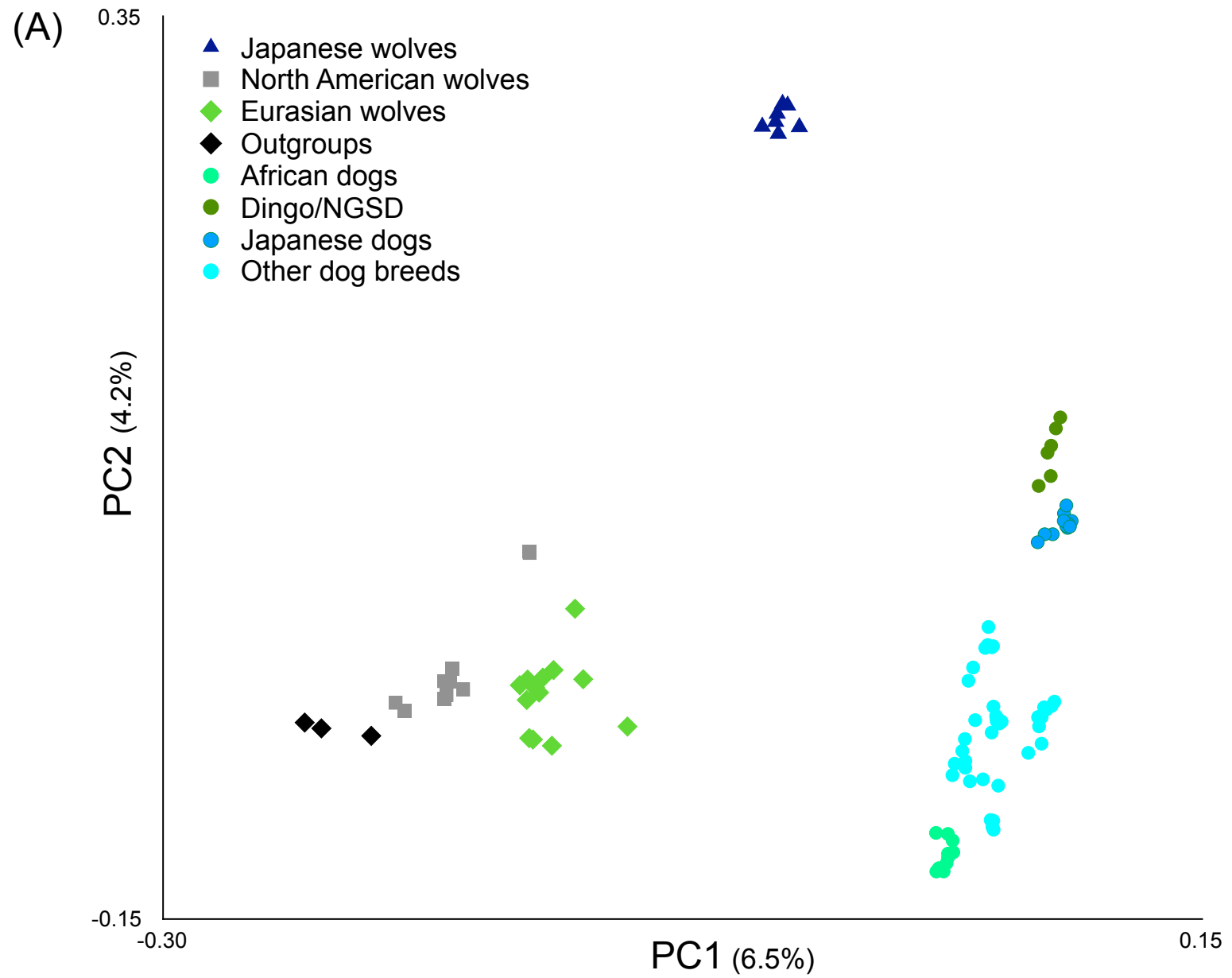
658   the overlapped region.

659

660

Figure 1 Relationships between Japanese wolves and other canids

(A) Principal Components Analysis (PC1 versus PC2) of 100 samples based on 2,065,200 SNPs (see Table S2 for sample information). Colored circle, square, and triangle correspond to the names of dogs or wolves in the panel. (B) ADMIXTURE results based on SNP data for K = 4 (see Table S2 for sample information).
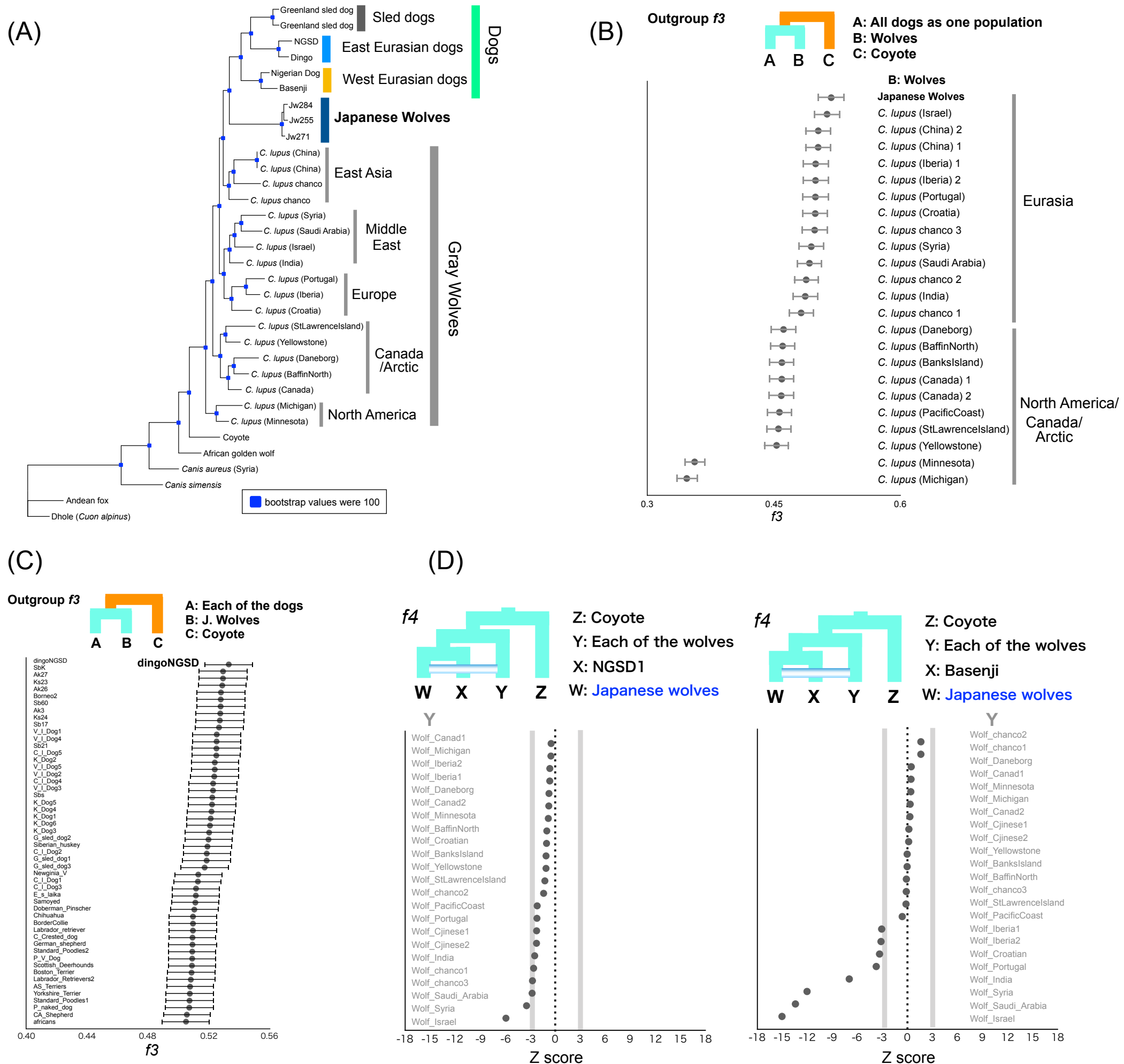
Figure 2 Phylogenetic relationships and genetic affinity between Japanese wolves and other canids.

(A) Maximum likelihood tree based on 1,971,890 SNPs. Node labels shown in blue squares indicate bootstrap values out of 100. (B) Shared genetic drift between dogs and gray wolves measured by outgroup $f3$ statistics. Each of all dogs and Japanese wolves were used as populations. Each $f3$ statistical value is plotted in order of highest to lowest value from the top, and the names of the wolves are shown on the right side of the panel. Error bars represent standard errors. (C) Shared genetic drift between Japanese wolf and all dogs measured by outgroup $f3$ statistics. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each $f3$ statistical value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the left side of the panel. Error bars represent standard errors. (D) $f4$ statistics testing the relationships between the Japanese wolf and all other wolves compared with NGSD1 (left panel) and Basenji (right panel). Each Z score is plotted in order of highest to lowest value from the top, and the names of wolves are shown on the left and right sides of the left and right panels, respectively. Gray lines show the Z score -3 and 3.
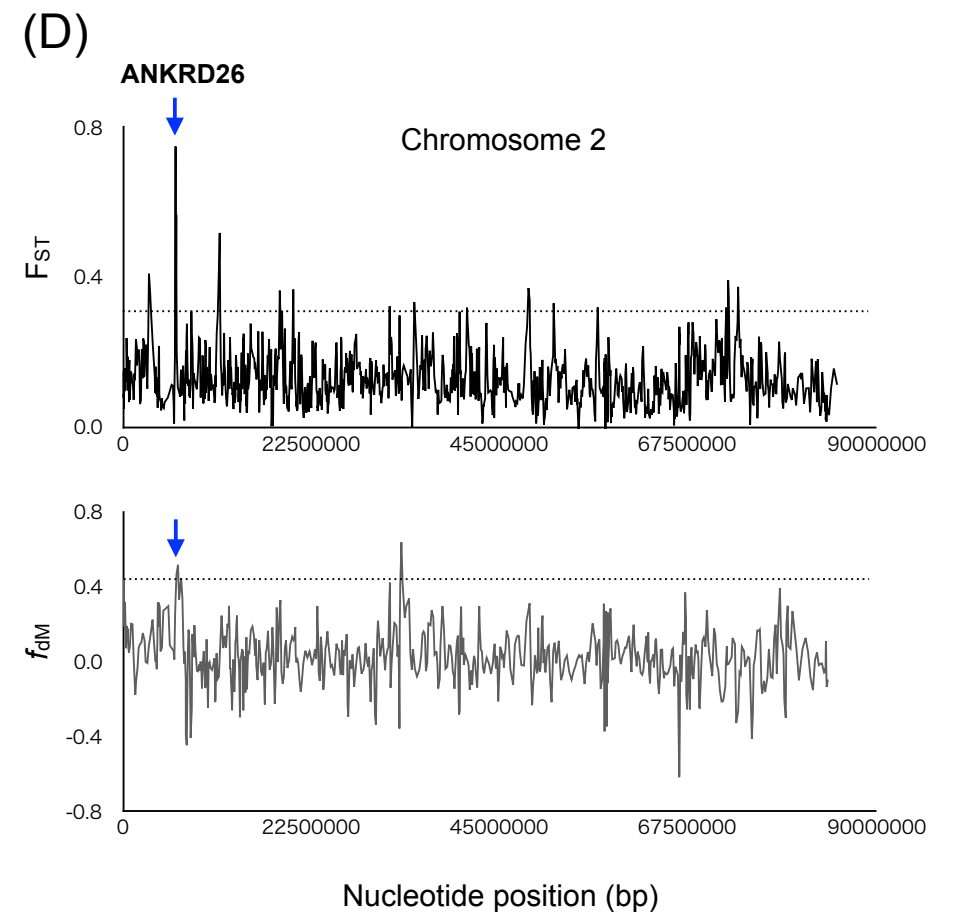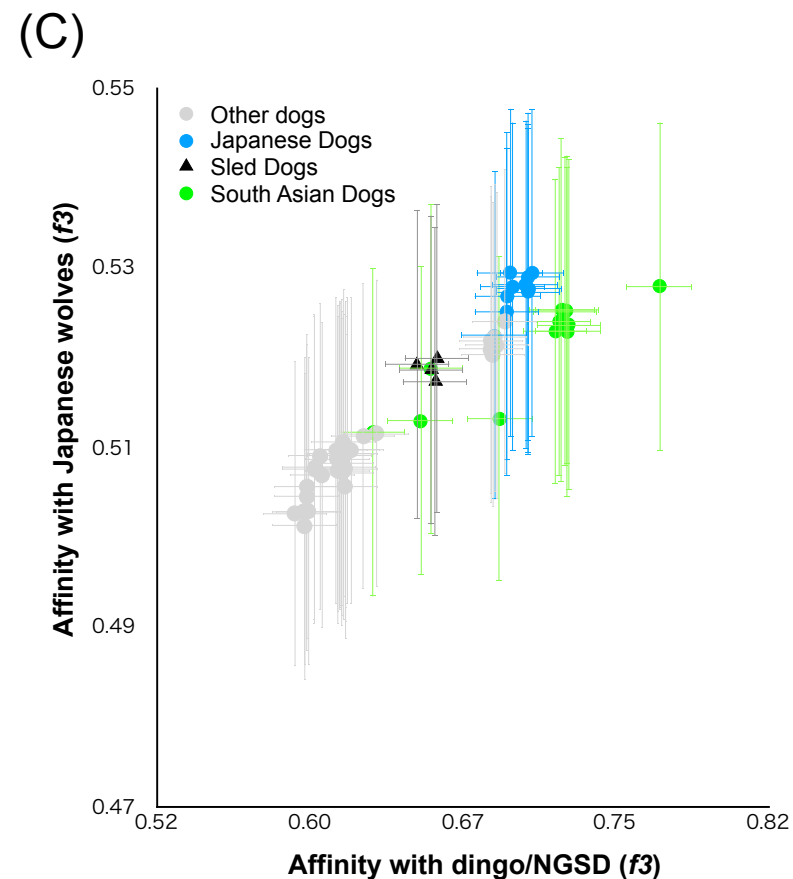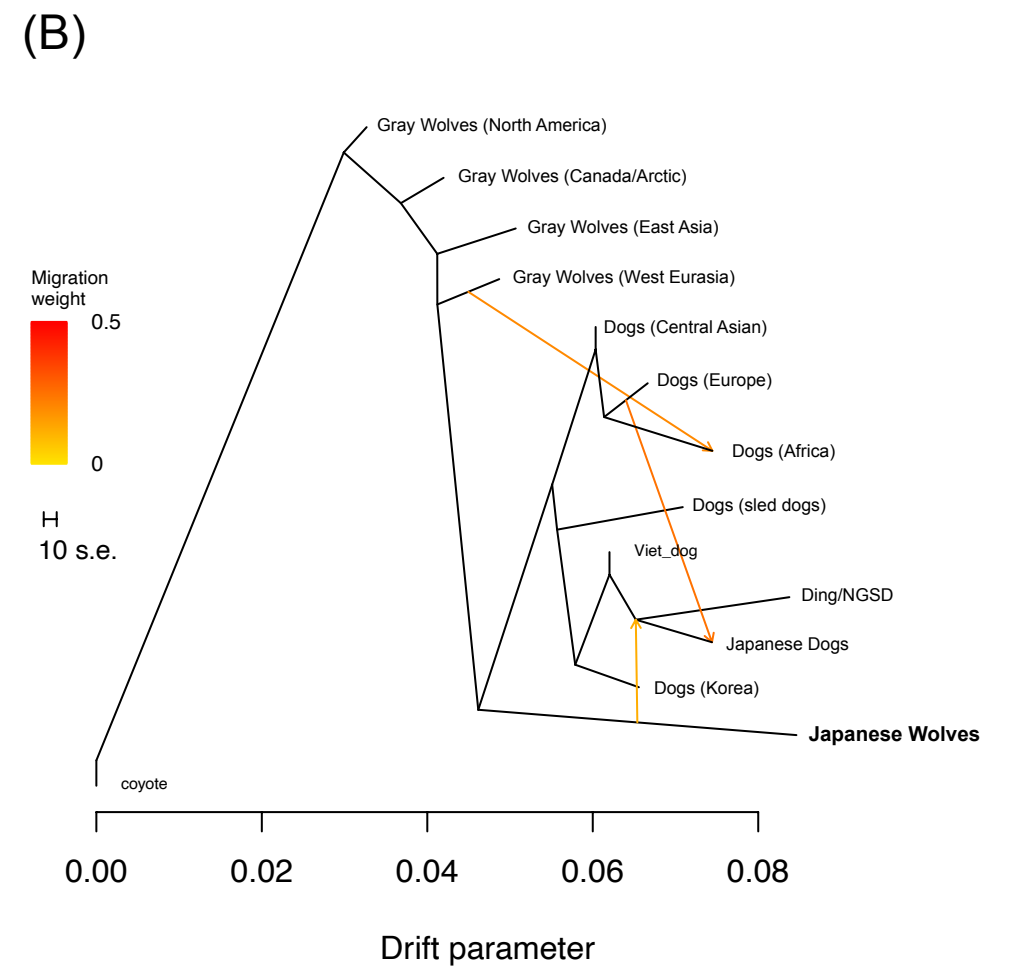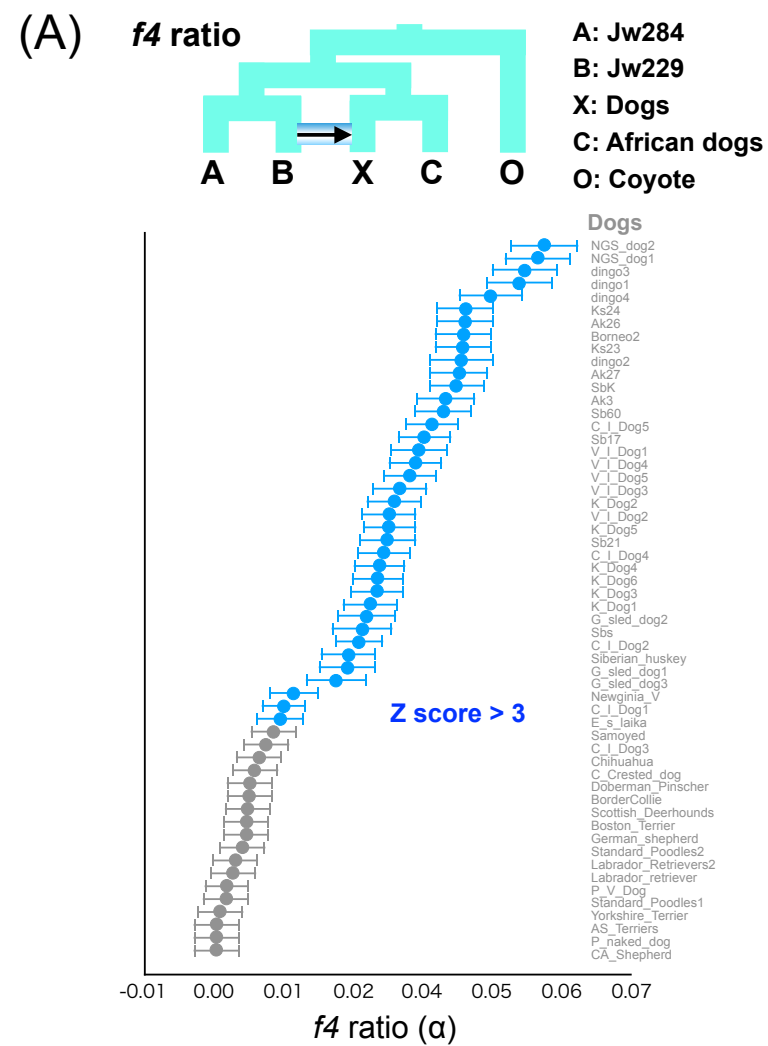
Figure 3 Admixture between Japanese wolves and the other canids.

f4-ratio test to estimate proportion of genome introgression from the Japanese wolf to dogs. Each *f4*-ratio α value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the right side of the panel. Error bars represent standard errors. Z score above 3 is colored in blue. (B) TreeMix admixture graph built using LD-pruned data (150,502 sites) on a dataset consisting of 88 dogs/wolves merged into 13 groups according to their phylogenetic relationships. (C) *f3* statistics testing whether dogs share more alleles with dingo/NGSD (x-axis) or Japanese wolf (y-axis). Dots show the *f3* statistics, and horizontal and vertical error bars represent standard errors for tests with the African dogs (x-axis) and dingo/NGSD (y-axis), respectively. Each of the Japanese wolves and dingo/NGSD individuals were used as populations. (D) Sliding window analyses of the F_{ST} values (y-axis: upper panel) and f_{dM} (y-axis: lower panel) in windows of 50 SNPs using a 25 SNPs slide across chromosome (x-axis). Dashed lines show the 99th percentiles. Blue arrow indicates overlapping regions above 99th percentiles between upper and lower panels. The gene name above the arrow is the gene in the overlapped region.
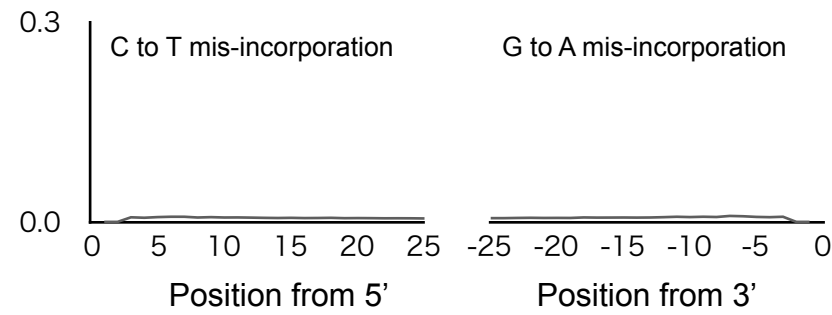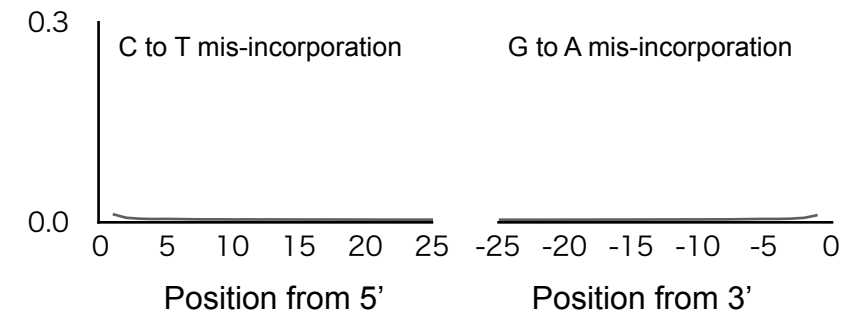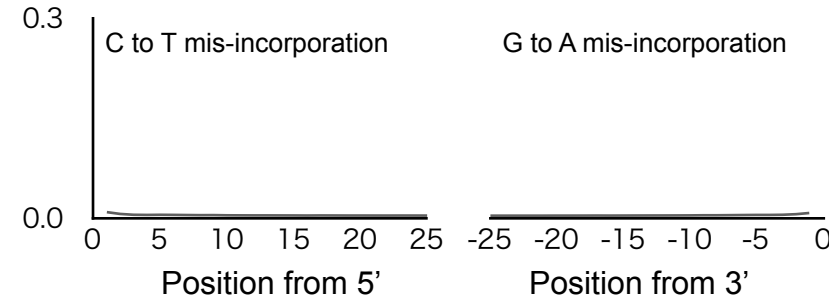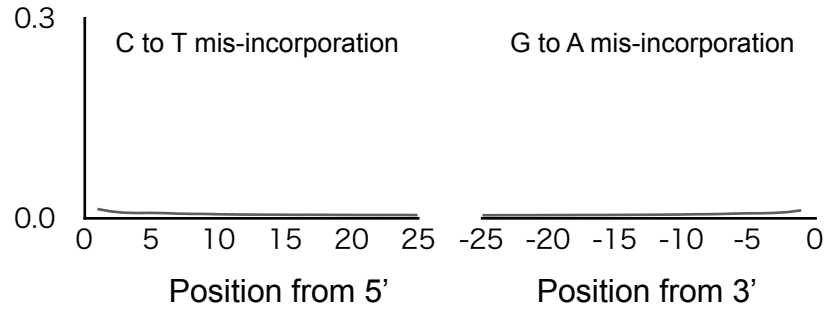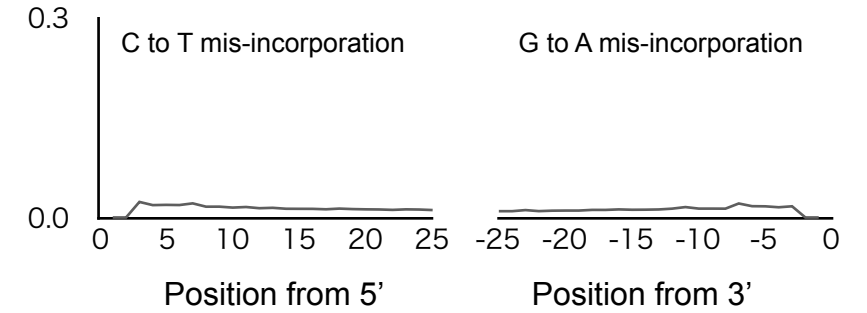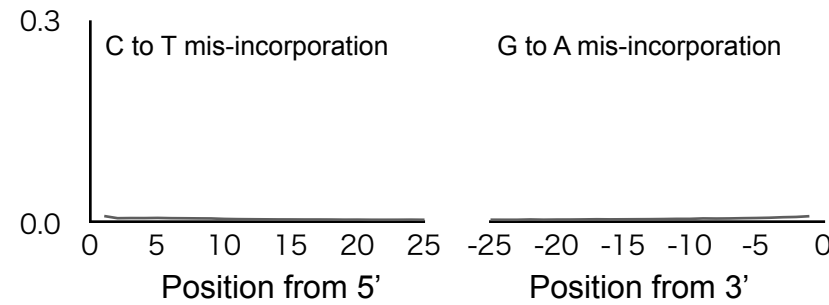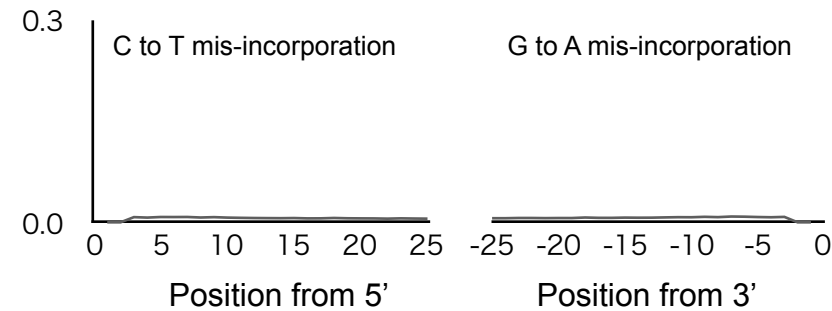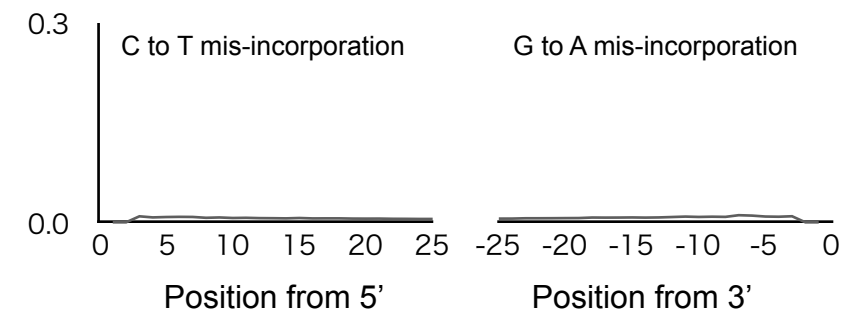
Figure S1

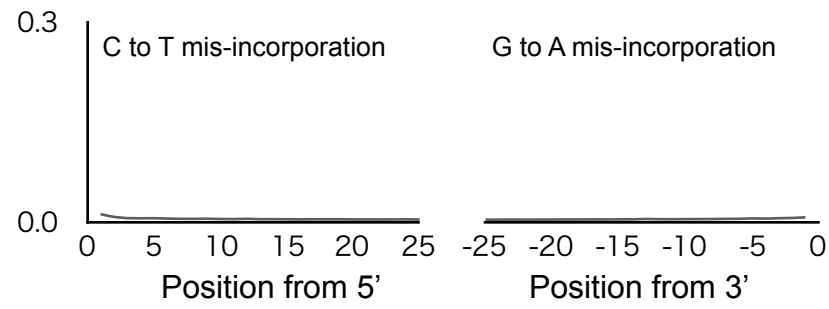C to T and G to A frequency of mis-incorporation at 3' and 5' end of reads determined in this study.
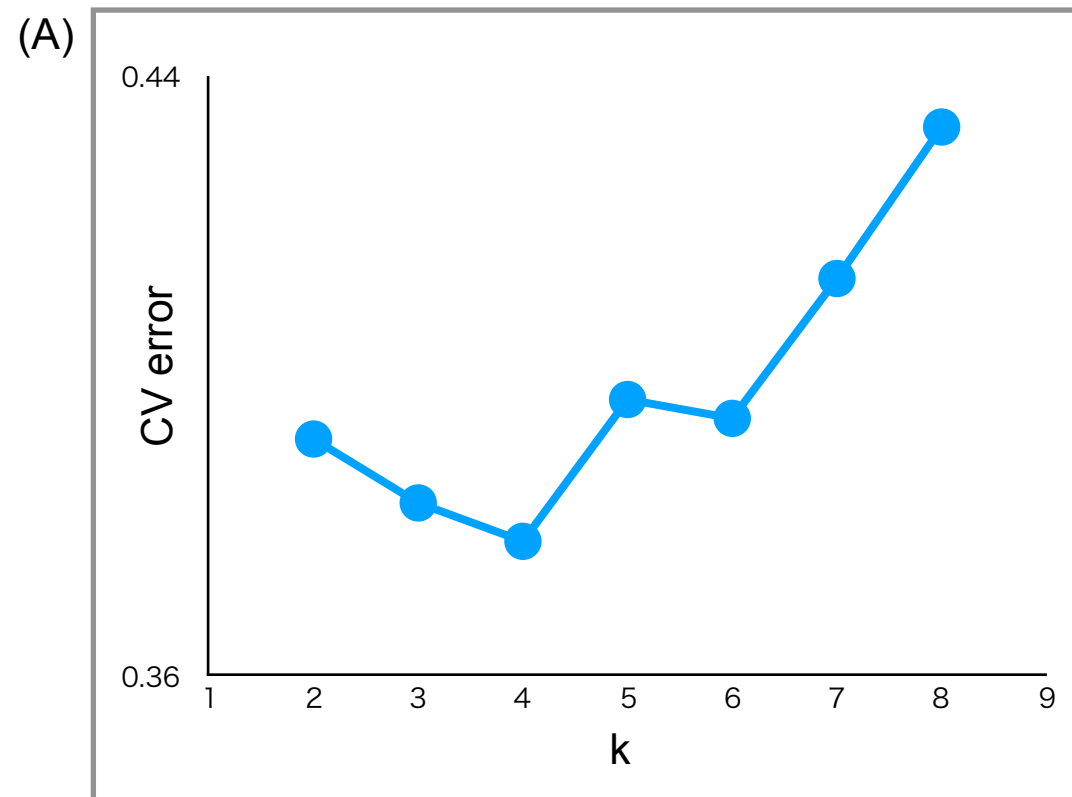
Figure S2
(A) Cross validation (CV) values for ADMIXTURE analysis of SNP data.
(B) ADMIXTURE results based on SNP data for K = 2-6 (see Table S2 for sample information).

(B)
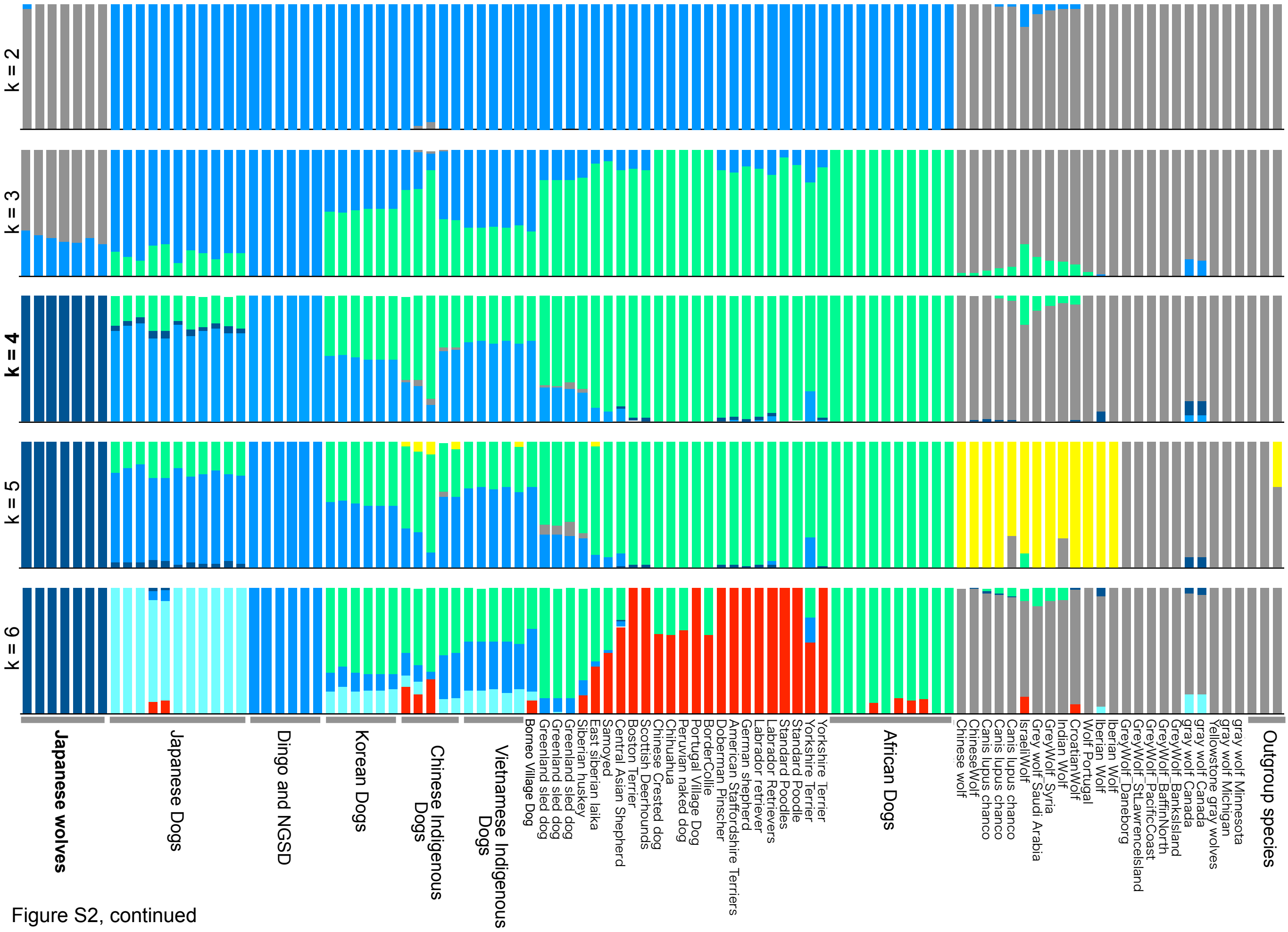
Figure S2, continued
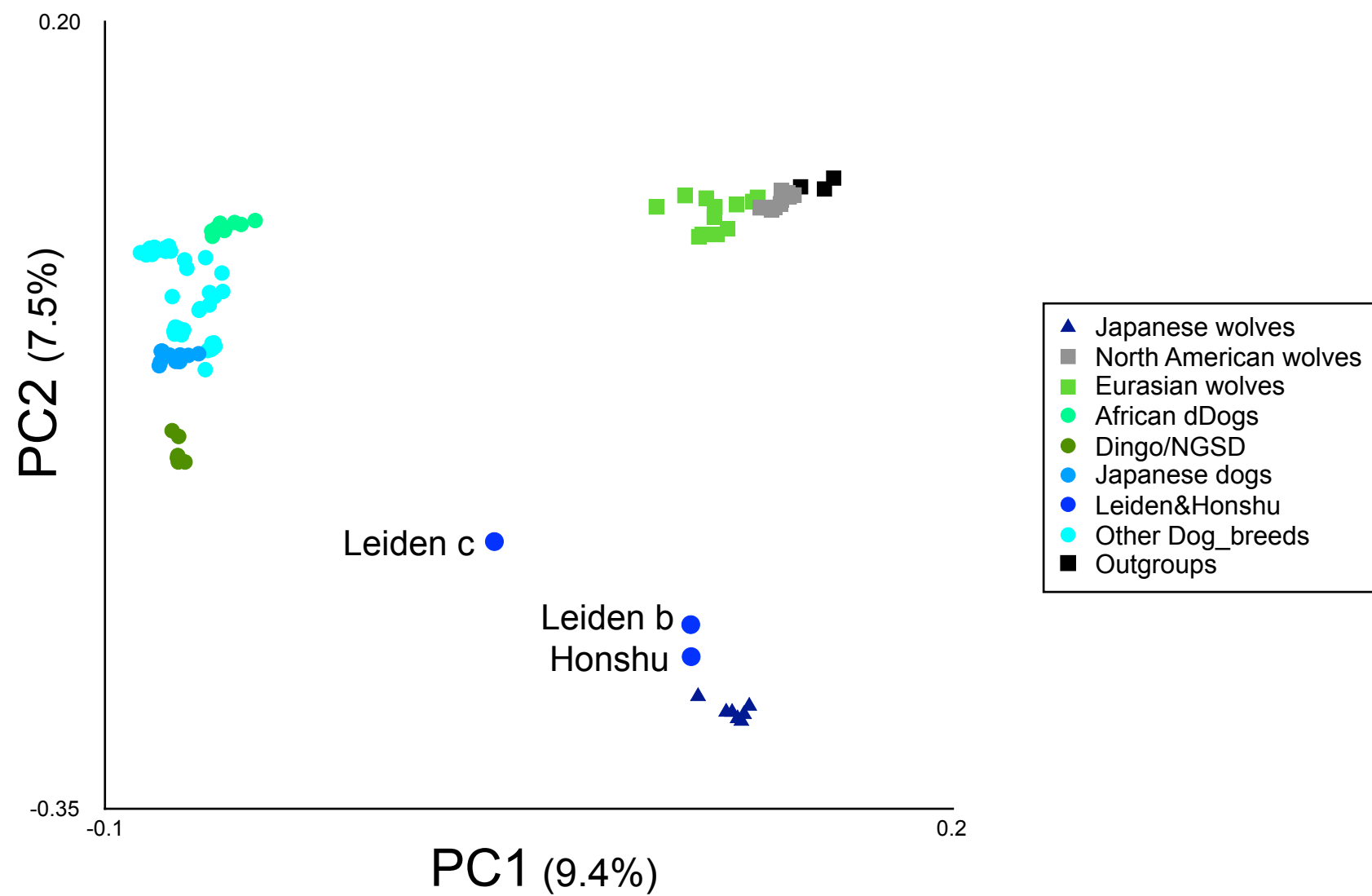
Figure S3
Principal Components Analysis (PC1 versus PC2) of 103 samples based on 103,432
SNPs (see Table S2 for sample information). Colored circle, square, and triangle
correspond to the names of dogs or wolves in the panel.

k = 4

Japanese wolves
Leiden c
Leiden b
Honshu
Japanese Dogs
Dingo and NGSD
Korean Dogs
Chinese Indigenous Dogs
Vietnamese Indigenous Dogs
Borneo Village Dog
Greenland sled dog
Greenland sled dog
Greenland sled dog
Siberian huskey
East siberian laika
Samoyed
Central Asian Shepherd
Boston Terrier
Scottish Deerhounds
Chinese Crested dog
Chihuahua
Peruvian naked dog
Portugal Village Dog
BorderCollie
Doberman Pinscher
American Staffordshire Terriers
German shepherd
Labrador retriever
Standard Poodles
Standard Poodle
Yorkshire Terrier
Yorkshire Terrier
African Dogs
Chinese wolf
ChineseWolf
Canis lupus chanco
Canis lupus chanco
Canis lupus chanco
IsraeliWolf
Grey wolf Saudi Arabia
GreyWolf Syria
Indian Wolf
CroatianWolf
Wolf Portugal
Iberian Wolf
Iberian Wolf
GreyWolf_Daneborg
GreyWolf_StLawrenceIsland
GreyWolf_PacificCoast
GreyWolf_BaffinNorth
GreyWolf BanksIsland
gray wolf Canada
gray wolf Canada
gray wolf Minnesota
gray wolf Michigan
Yellowstone gray wolves
Outgroup species

Figure S4
An ADMIXTURE result based on SNP data for K = 4 (see Table S2
for sample information). Cross validation (CV) values for
ADMIXTURE analysis of SNP data is shown in the panel.
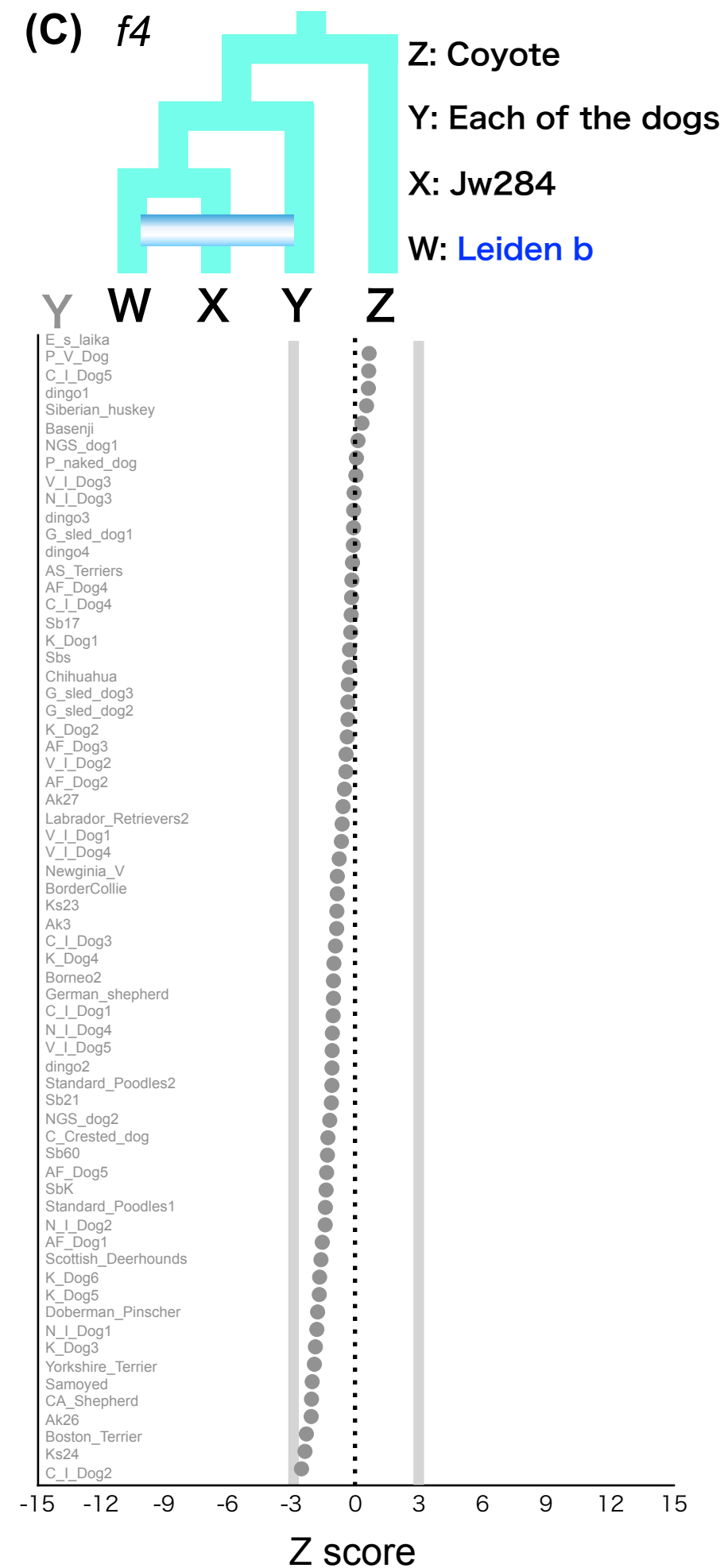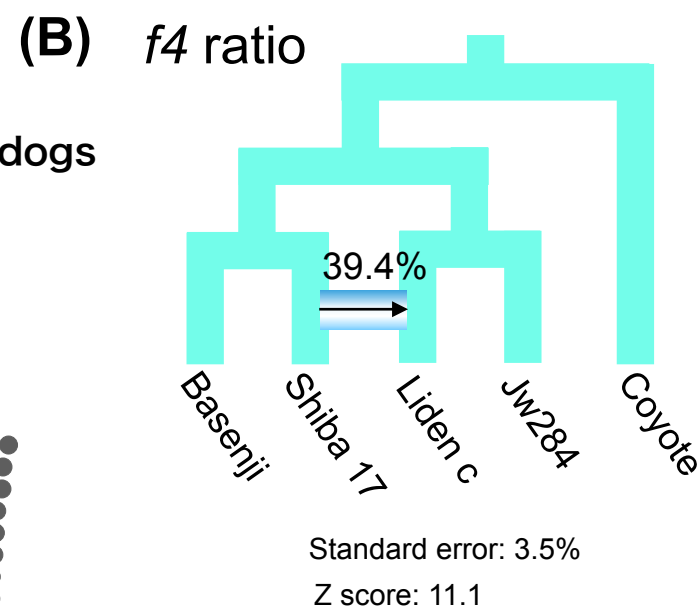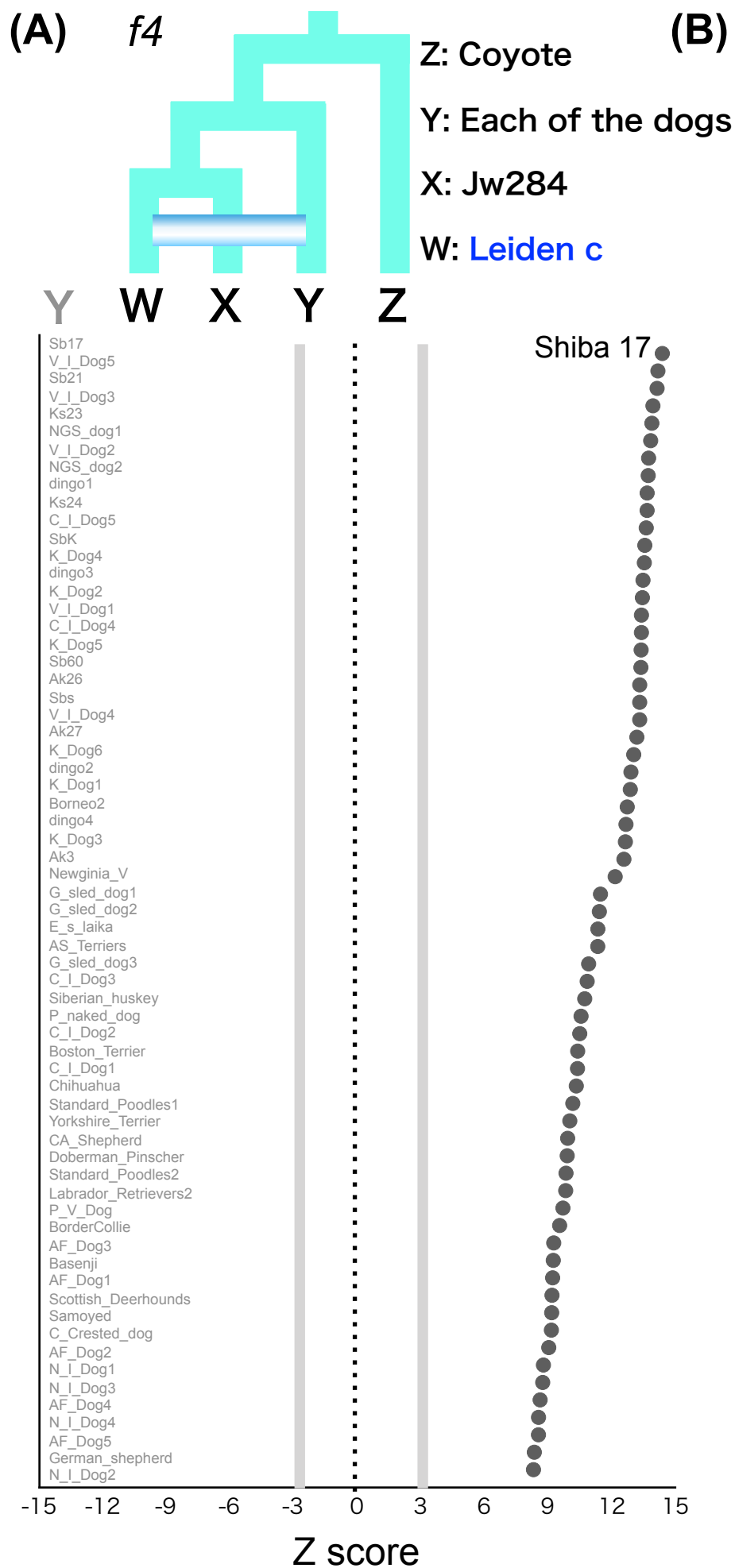
Figure S5
(A) *f4*-statistics testing the relationships between the Japanese wolf (Jw284), Leiden c, and all other dogs. Each Z score is plotted in order of highest to lowest value from the top, and the names of dogs are shown on the left side of each panel. Gray lines show the Z score -3 and 3. Leiden c shows genetic affinity with all other dogs (Z score > 3). (B) *f4*-ratio test to estimate proportion of genome introgression from a Japanese dog (Shiba 17) to Leiden C. (C) *f4*-statistics testing the relationships between the Japanese wolf (Jw284), Leiden b, and all other dogs. The genetic affinities of Leiden b with all other dogs are rejected (-3 < Z score < 3).
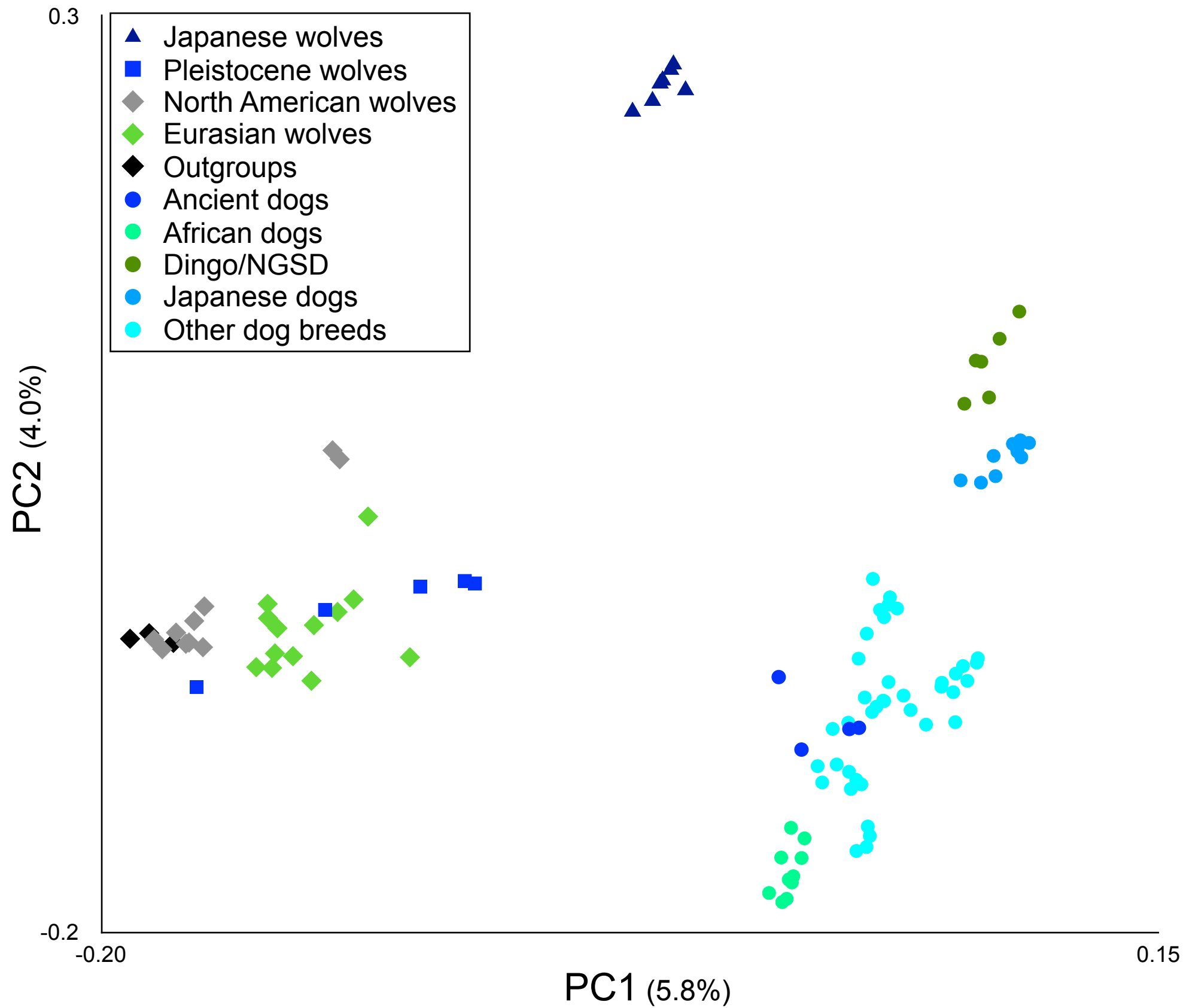
Figure S6

Principal Components Analysis (PC1 versus PC2) of 109 samples based on 100,588 SNPs (transversion sites, see Table S2 for sample information). Colored circle, square, and triangle correspond to the names of dogs or wolves in the panel.

Figure S7
Maximum likelihood tree
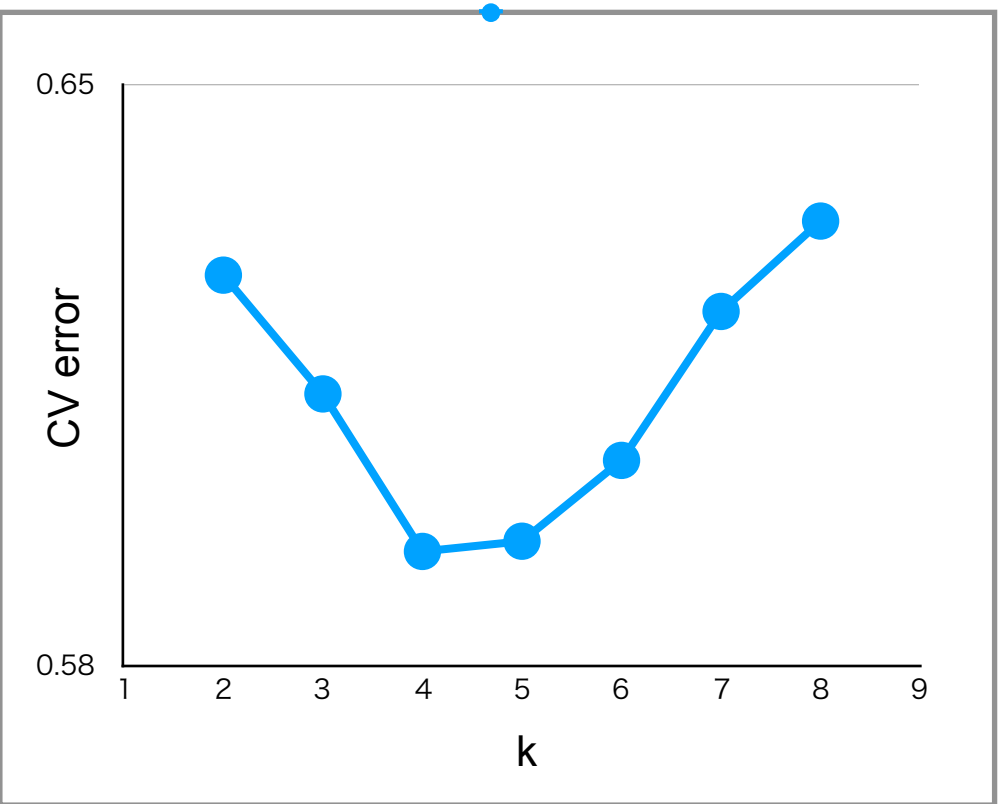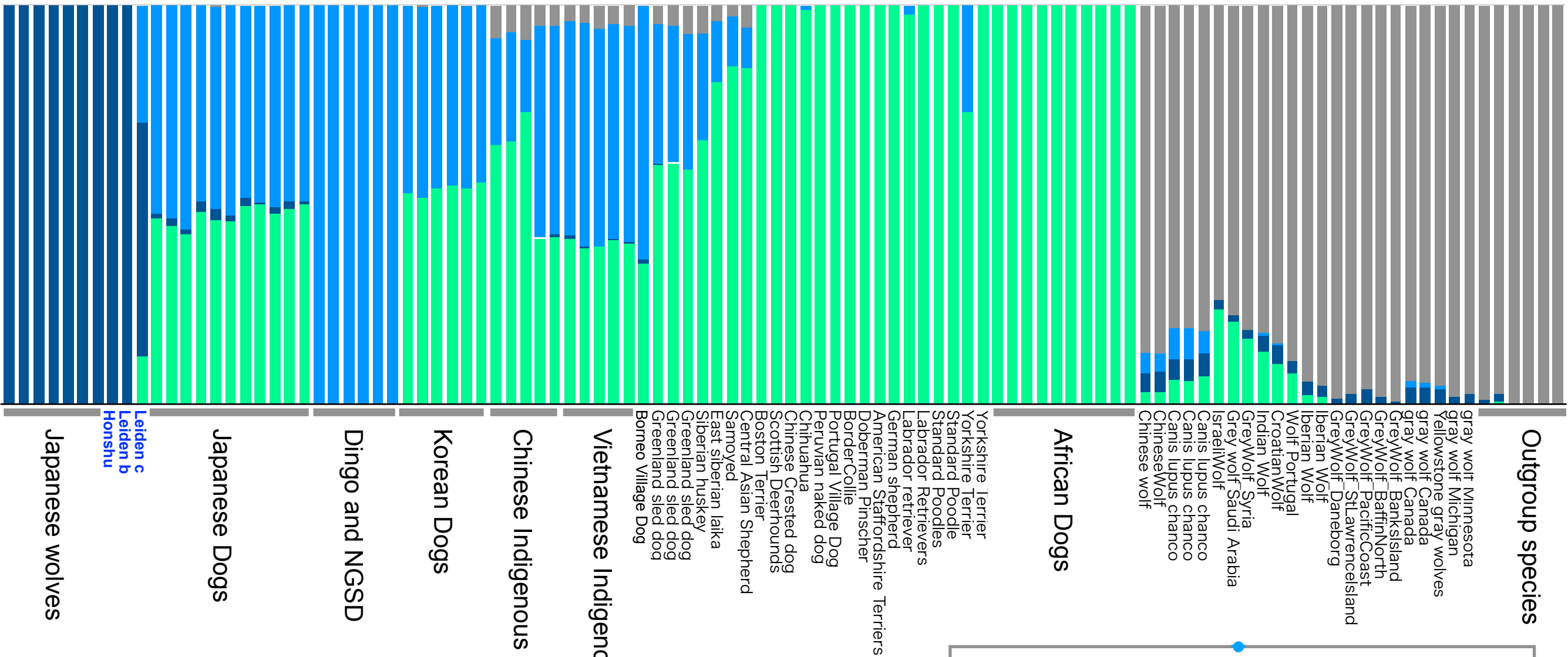based on 489,524 SNPs.
Node labels indicate
bootstrap replicates.
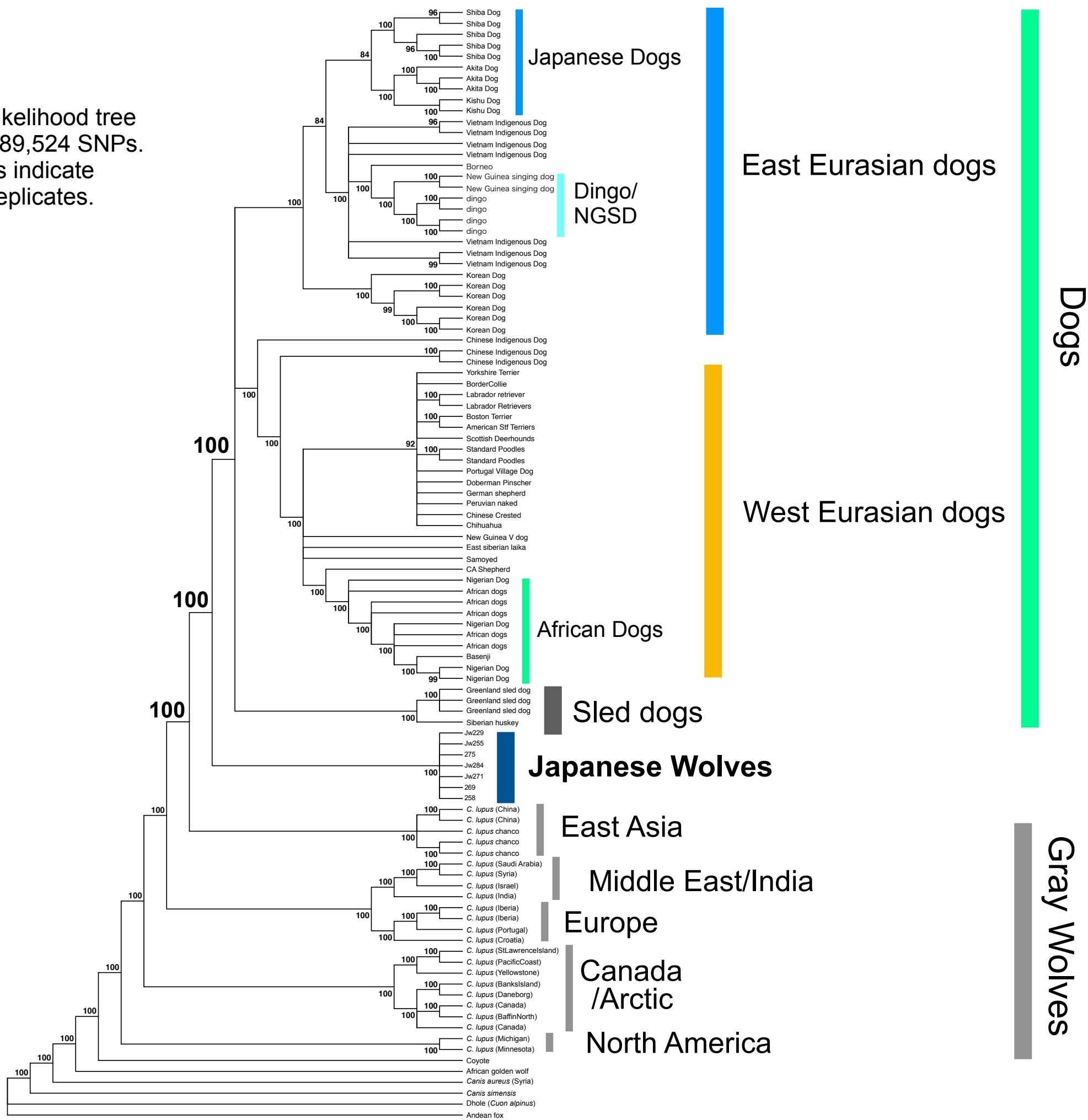
Figure S8
Phylogenetic tree constructed by SVDquartets based on 489,524 SNPs. Node labels indicate bootstrap replicates.
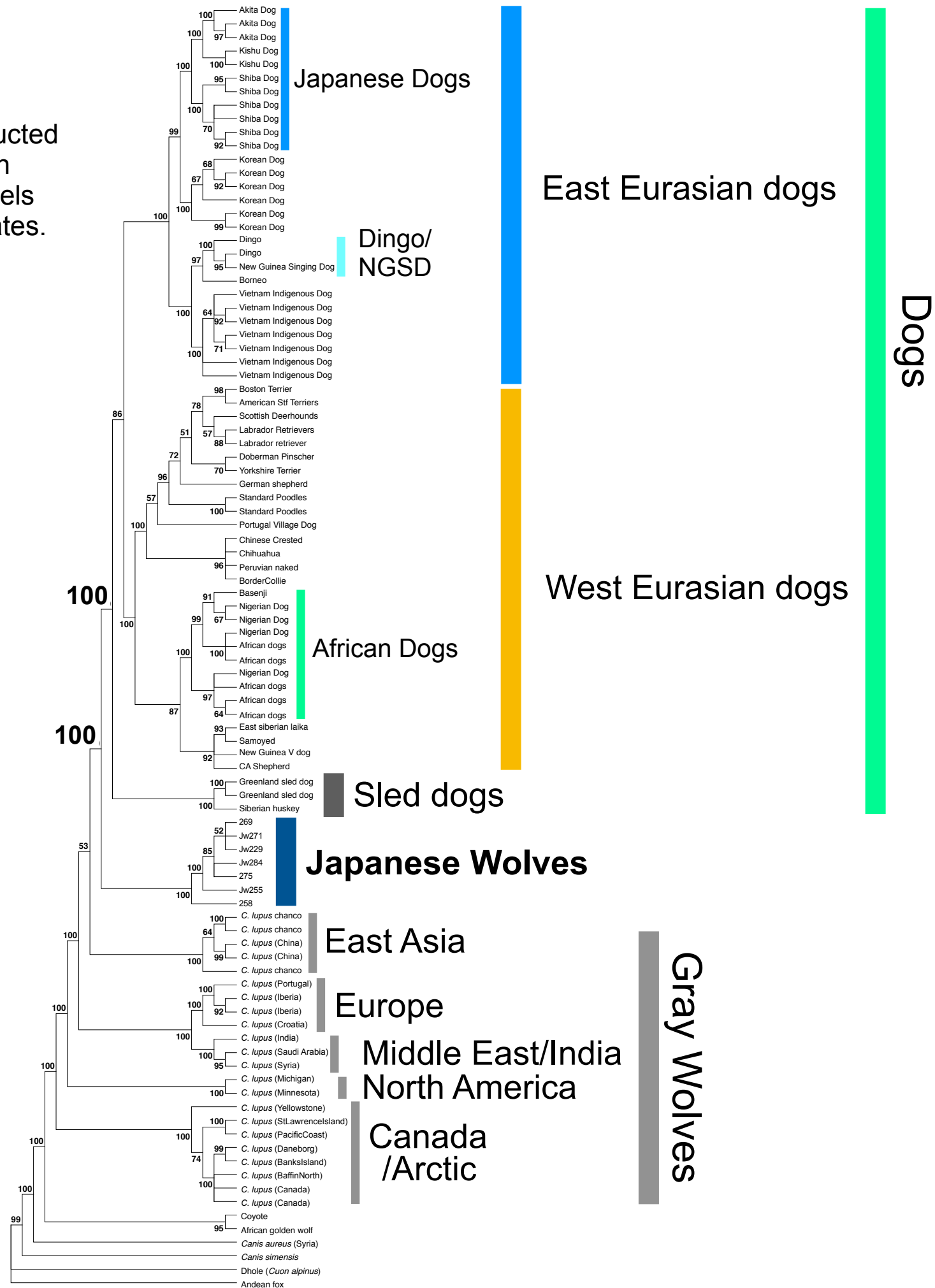
Figure S9
NJ tree based on IBS matrix
from 1,992,260 SNPs.
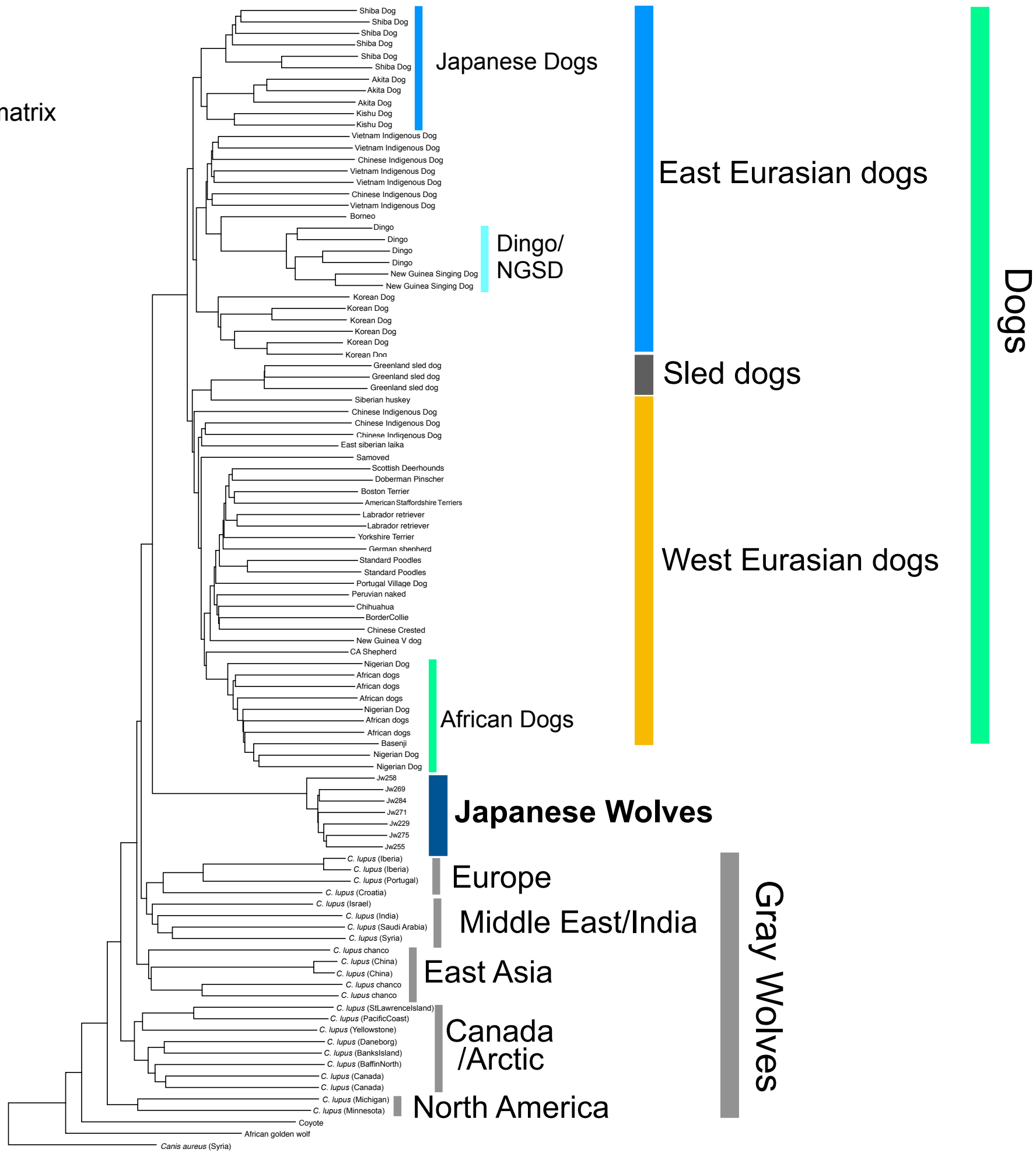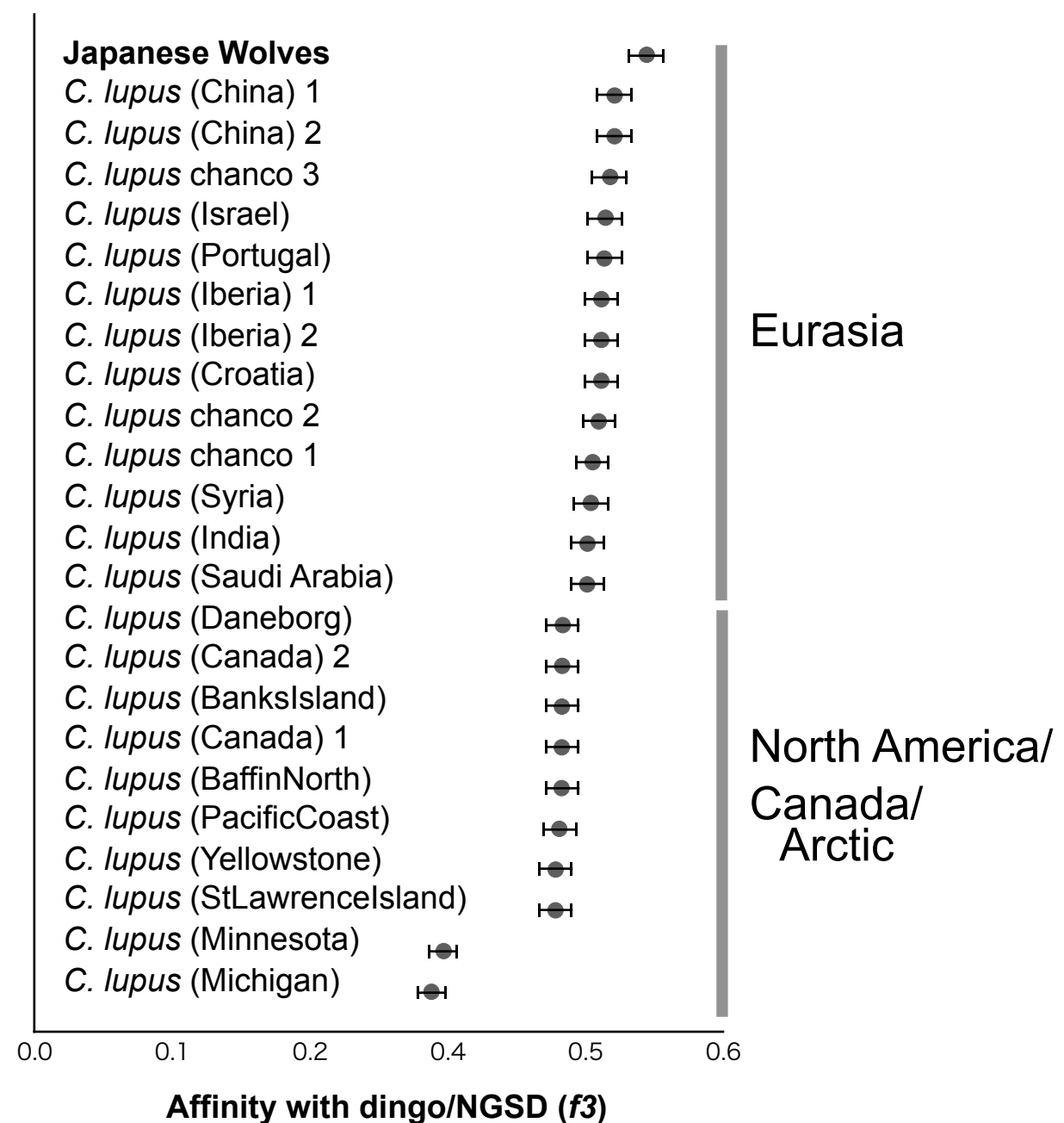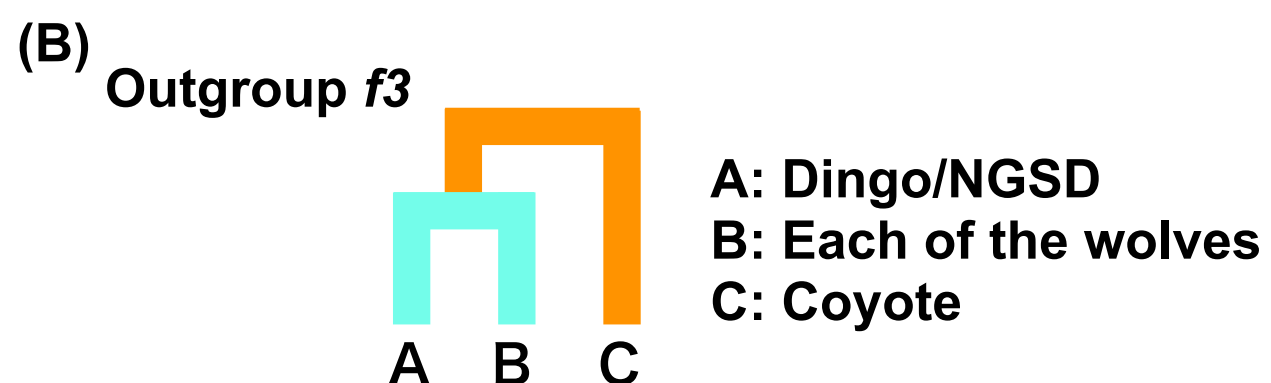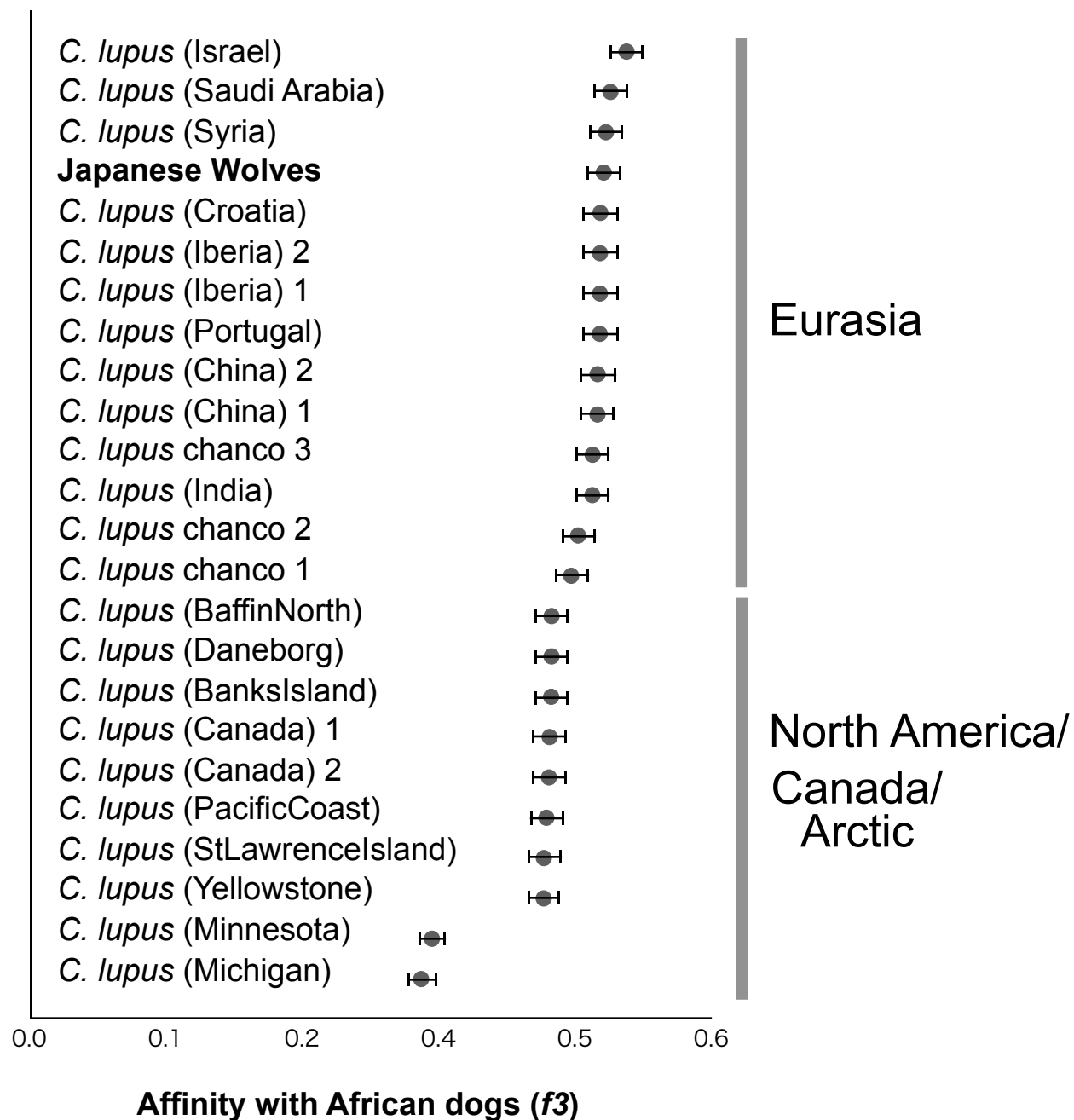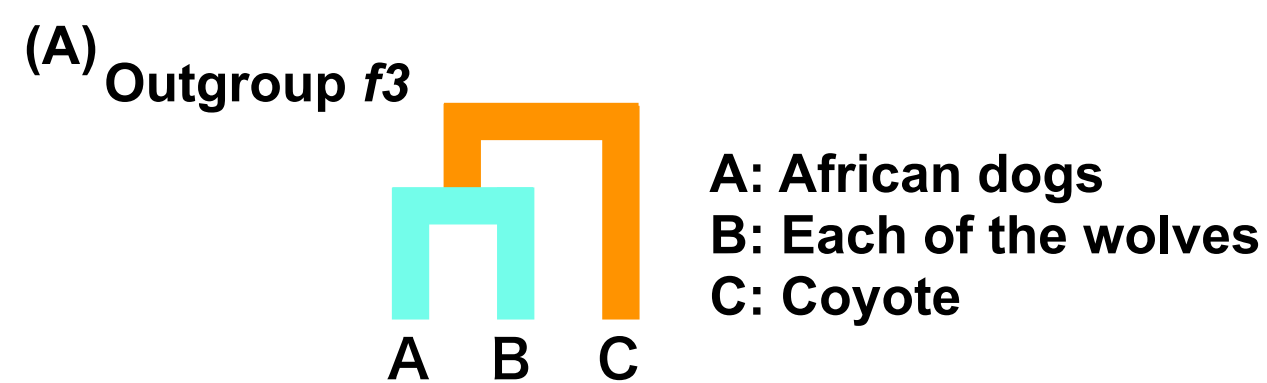
Figure S10

Shared genetic drift between African dogs (A) and Dingo/NGSD (B) and gray wolves measured by outgroup *f3* statistics. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each *f3* statistical value is plotted in order of highest to lowest value from the top, and the names of the wolves are shown on the left side of each panel. Error bars represent standard errors.

Figure S11

Figure S11
f4 statistics testing the genetic affinity of the Japanese wolf with all other dogs. All Japanese wolves were used as a population. Z scores for each combination are plotted. We computed *f4* statistics where W in the schematic representation is shown and fixed in each panel and X represented any possible other dogs. Each Z score is listed in order of highest to lowest value from the top. Dotted line shows the Z score 3. Large size graphs of highest (NGS_dog1) and lowest (Nigerian Dog2) affinity to the Japanese wolf are show in the first two panels. The names of the dogs are shown on the left or right sides of the panels. Small size panels of these two are surrounded by gray squares.

f4
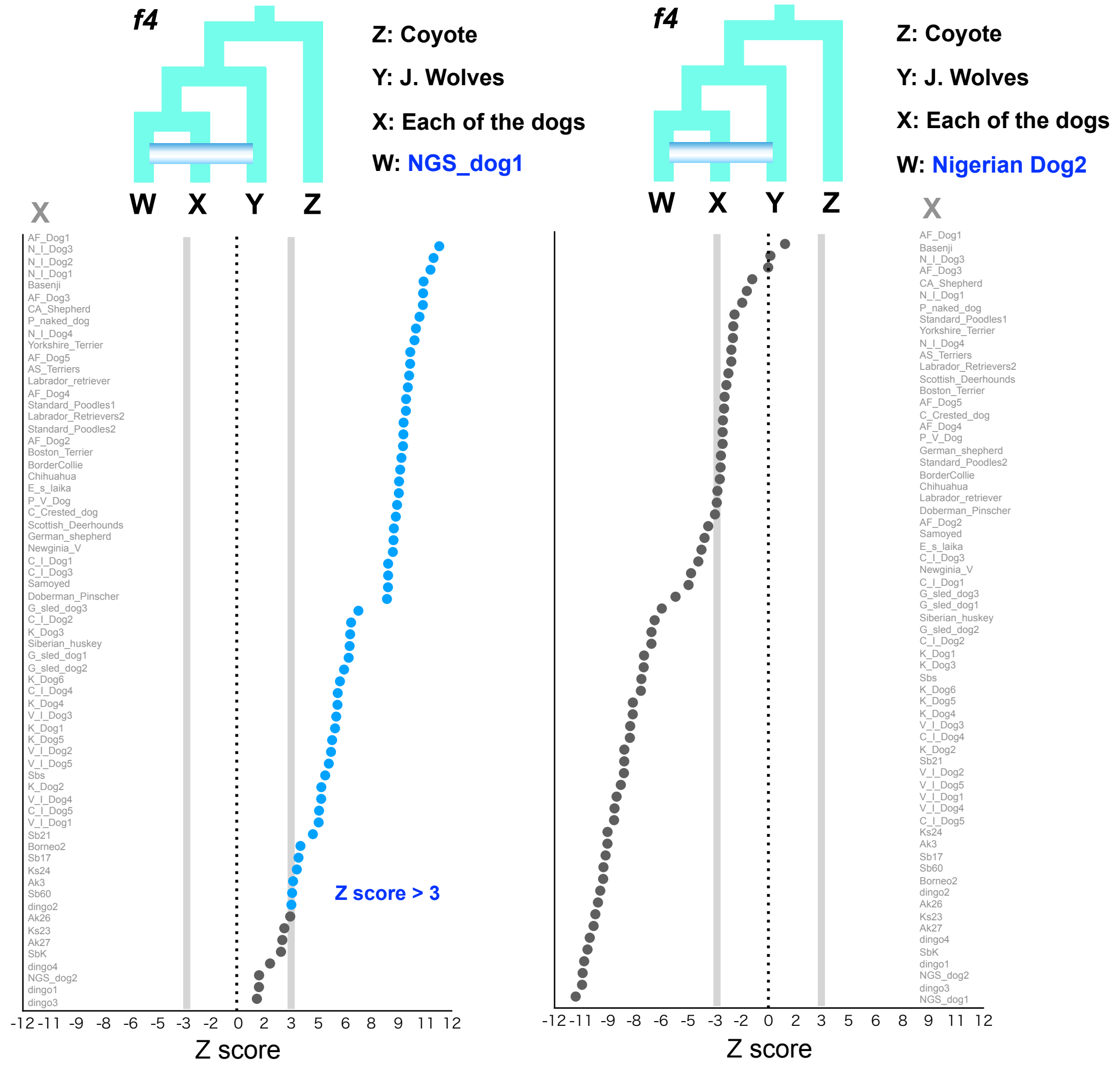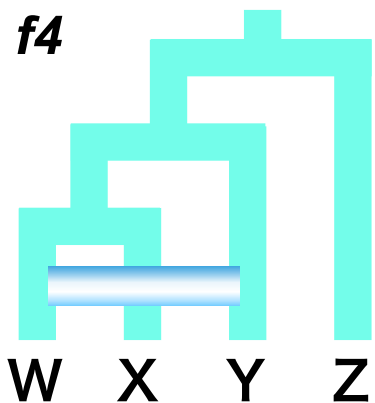
Z: Coyote

Y: J. Wolves

X: Each of the dogs

W: Dog shown in the panel
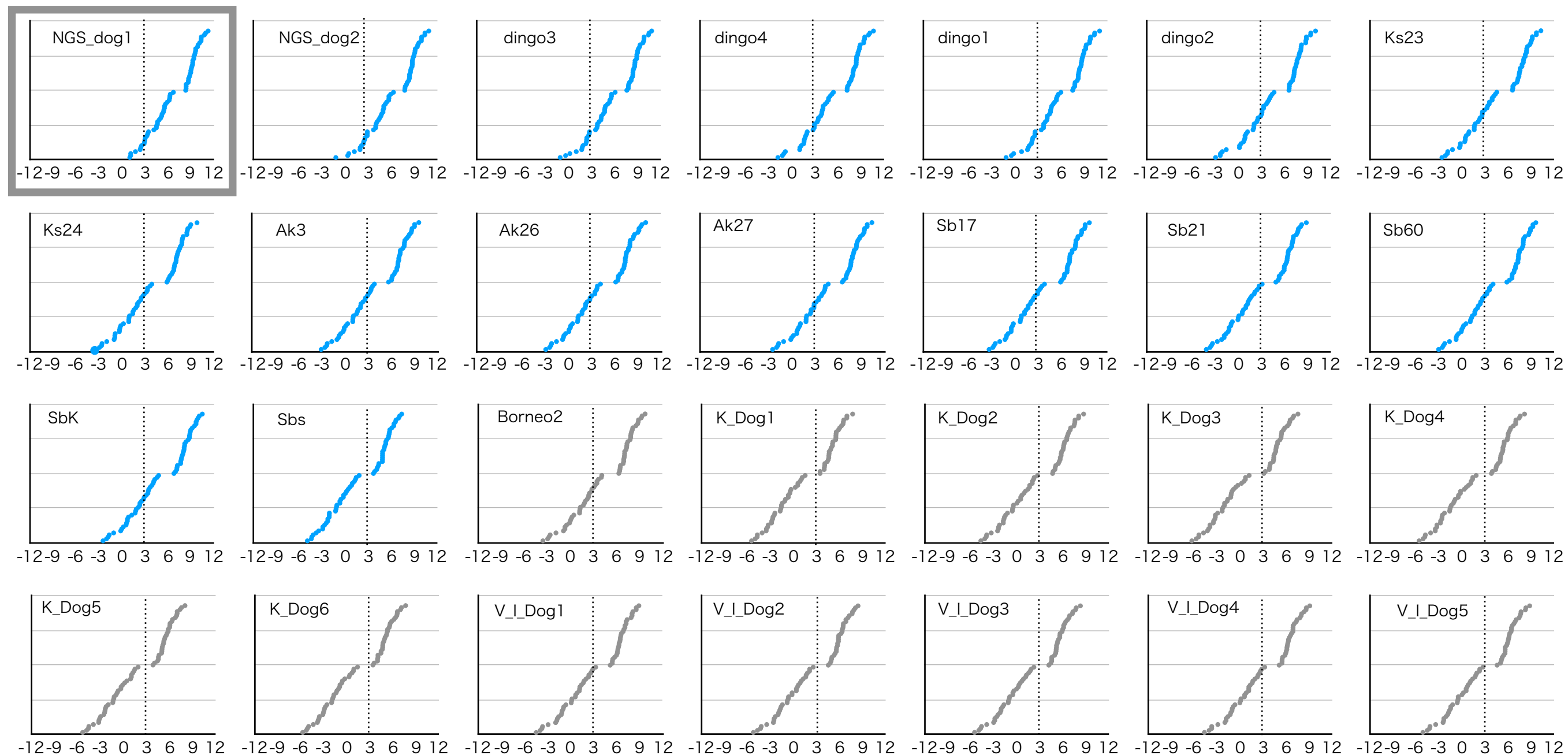
Figure S11 continued
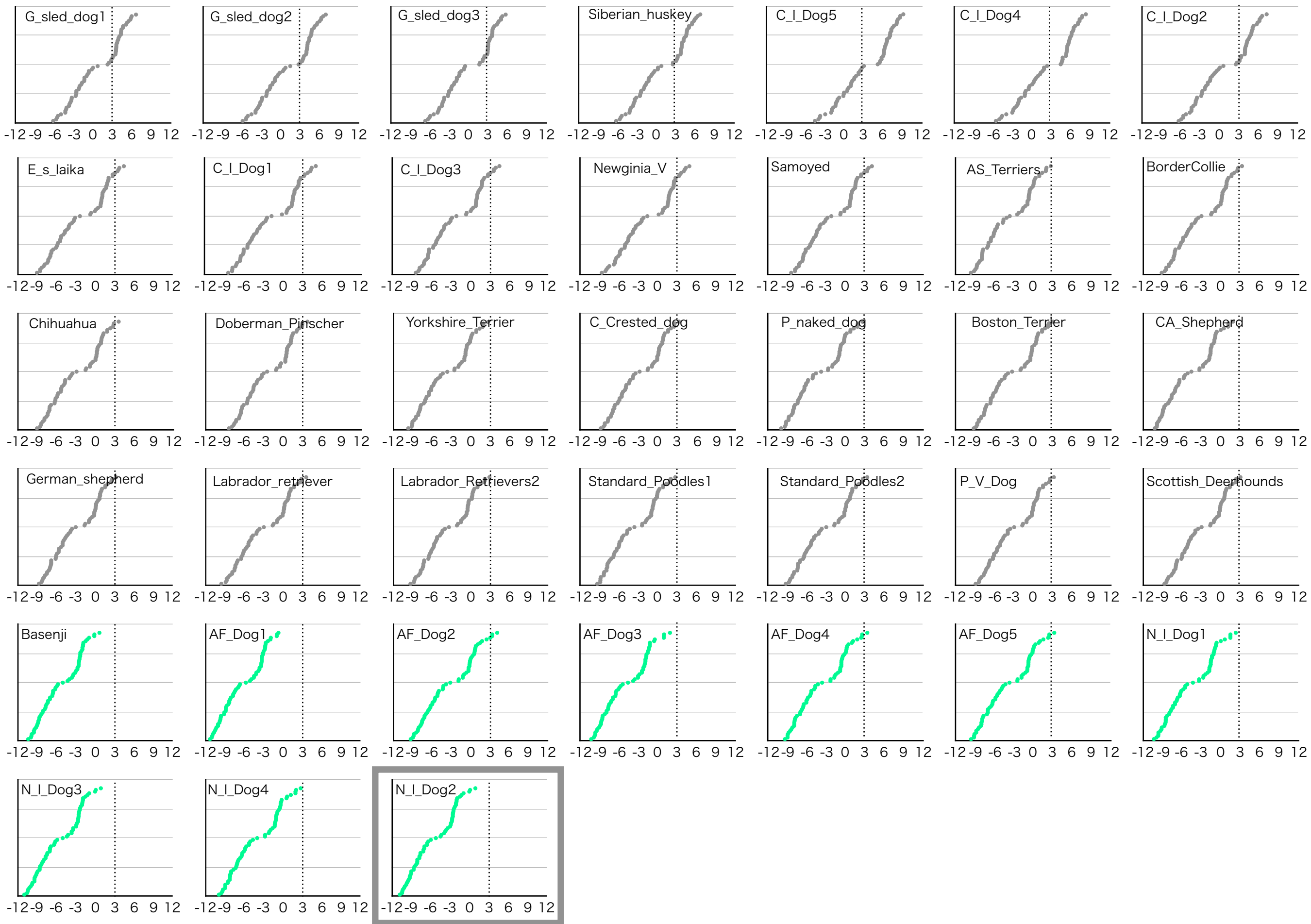
Z score

Figure S11 continued

Z score

North America Canada/Arctic

Wolf_Michigan, Wolf_Minnesota, Wolf_BaffinNorth, Wolf_BanksIsland, Wolf_Canad1, Wolf_Canad2, Wolf_Daneborg, Wolf_PacificCoast, Wolf_StLawrenceIsland, Wolf_Yellowstone

f4

W X Y Z
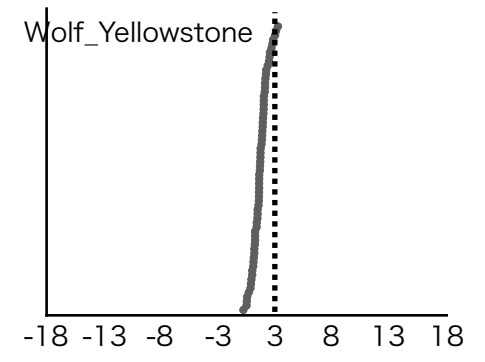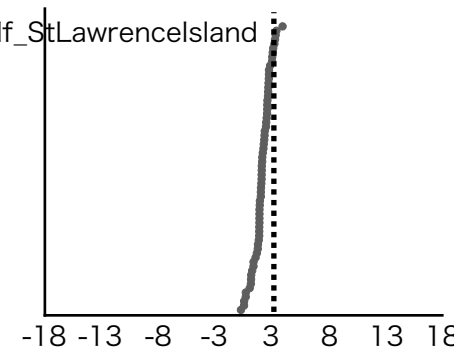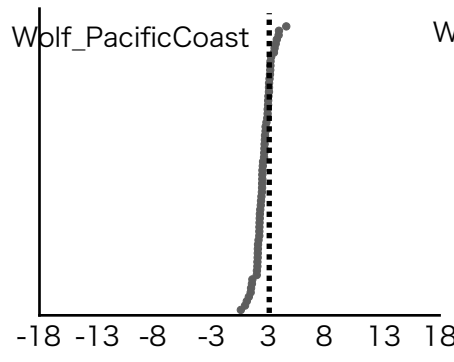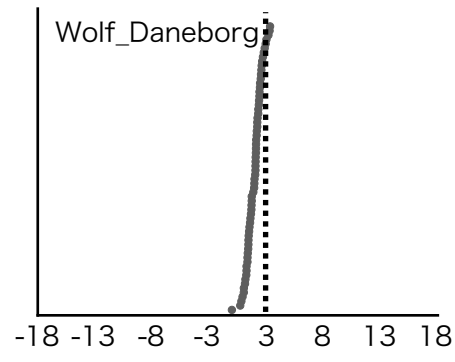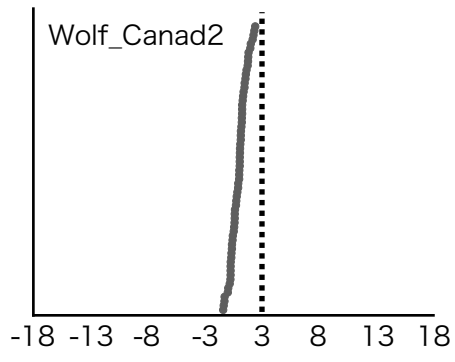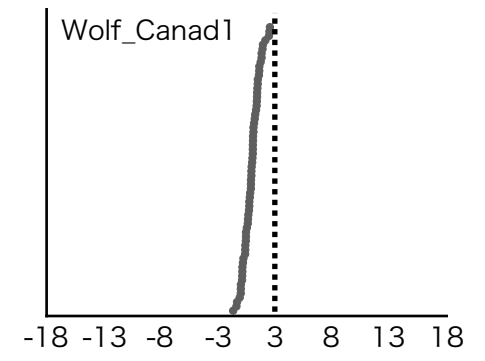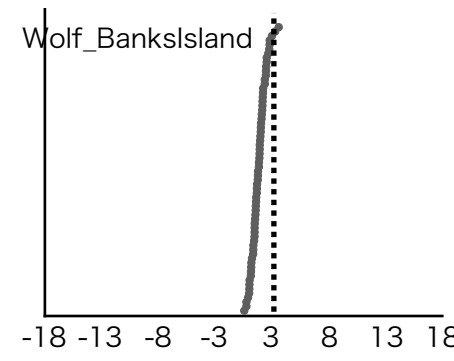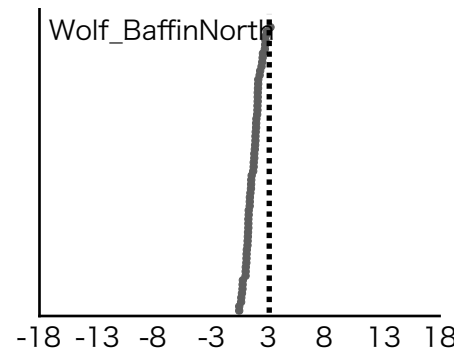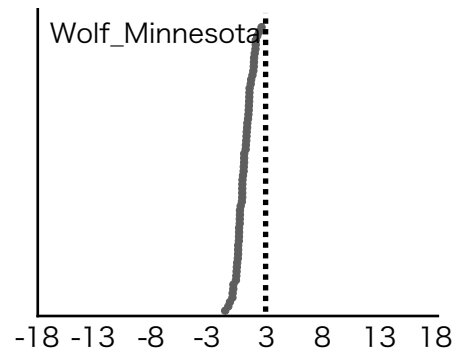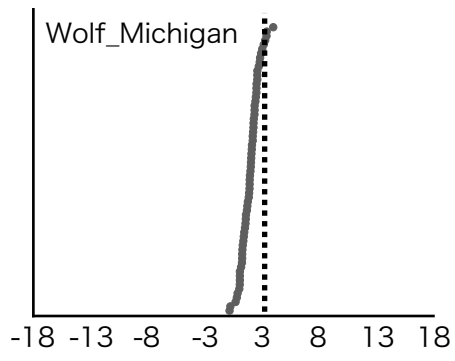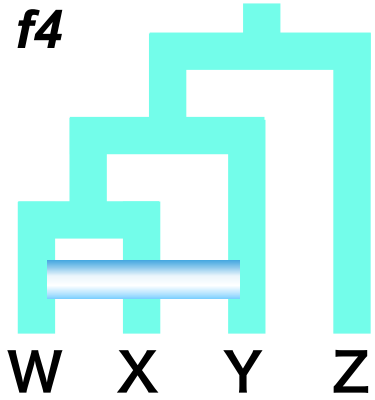
Z: Coyote
Y: Wolves shown in the panel
X: Japanese Wolves
W: Each of the dogs

Europe

Wolf_Croatian, Wolf_Iberia1, Wolf_Iberia2, Wolf_Portugal

Middele East India

Wolf_India, Wolf_Israel, Wolf_Saudi Arabia, Wolf_Syria

East Asia
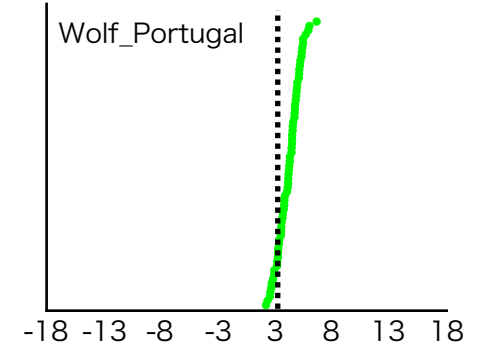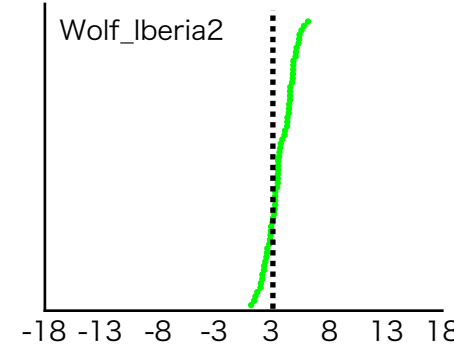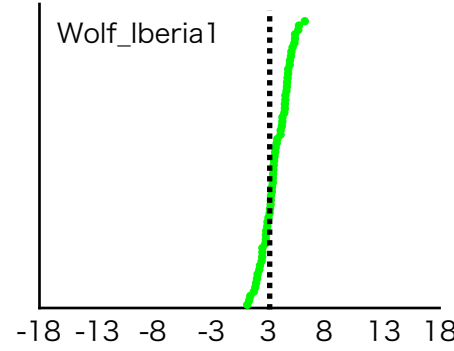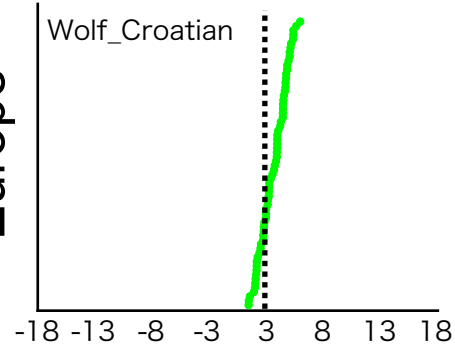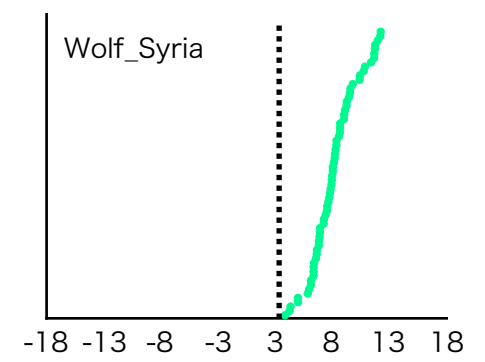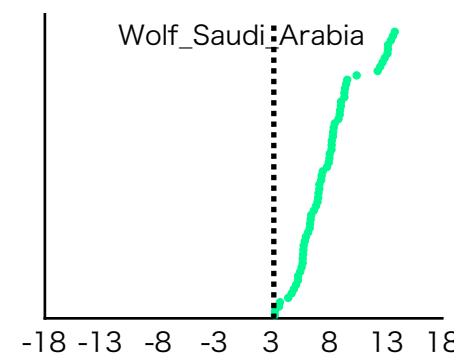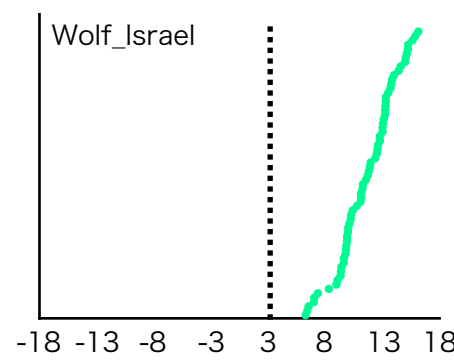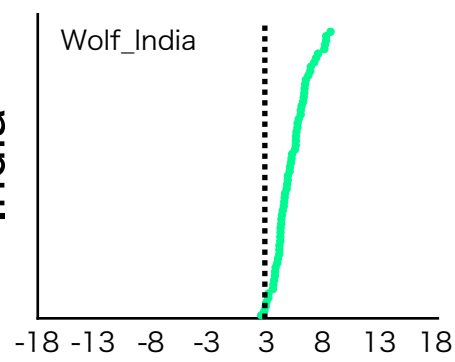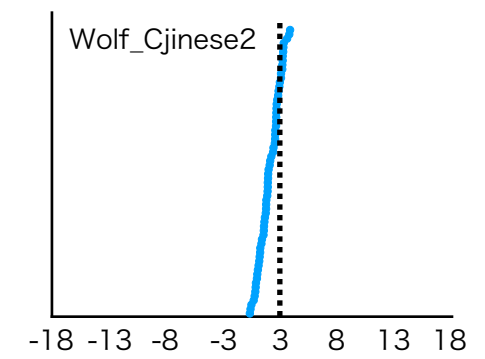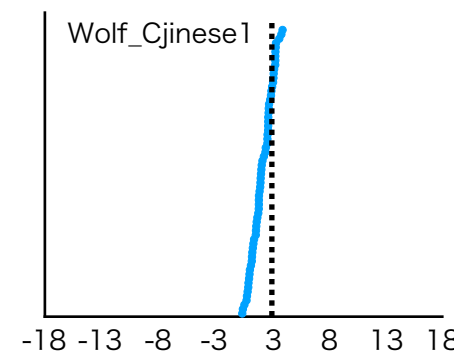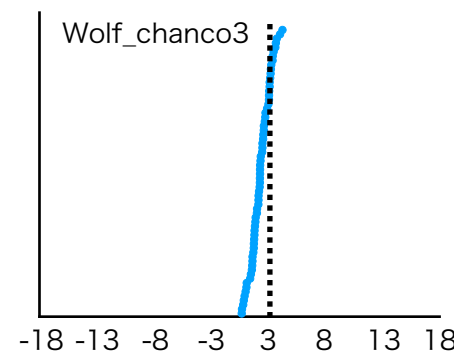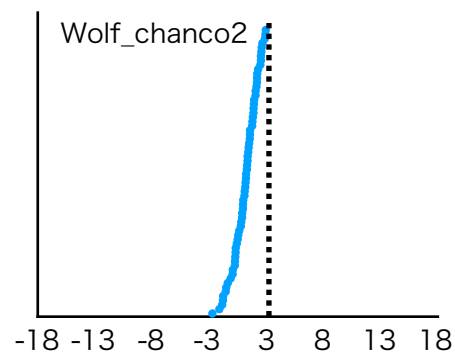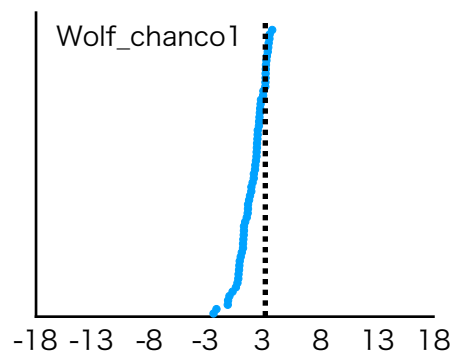
Wolf_chanco1, Wolf_chanco2, Wolf_chanco3, Wolf_Cjinese1, Wolf_Cjinese2

Z score

Figure S12

Figure S12

f4 statistics testing the genetic affinity of gray wolves shown in each panels with all dogs. Z scores for each combination are plotted in order of highest to lowest value from the top. We computed *f*4 statistics where Y in the schematic representation is shown and fixed in each panel and W represented any possible other dogs. Dotted line shows the Z score 3.

Figure S13
Maximum likelihood tree based on 2,065,200 SNPs. Node labels indicate bootstrap replicates.
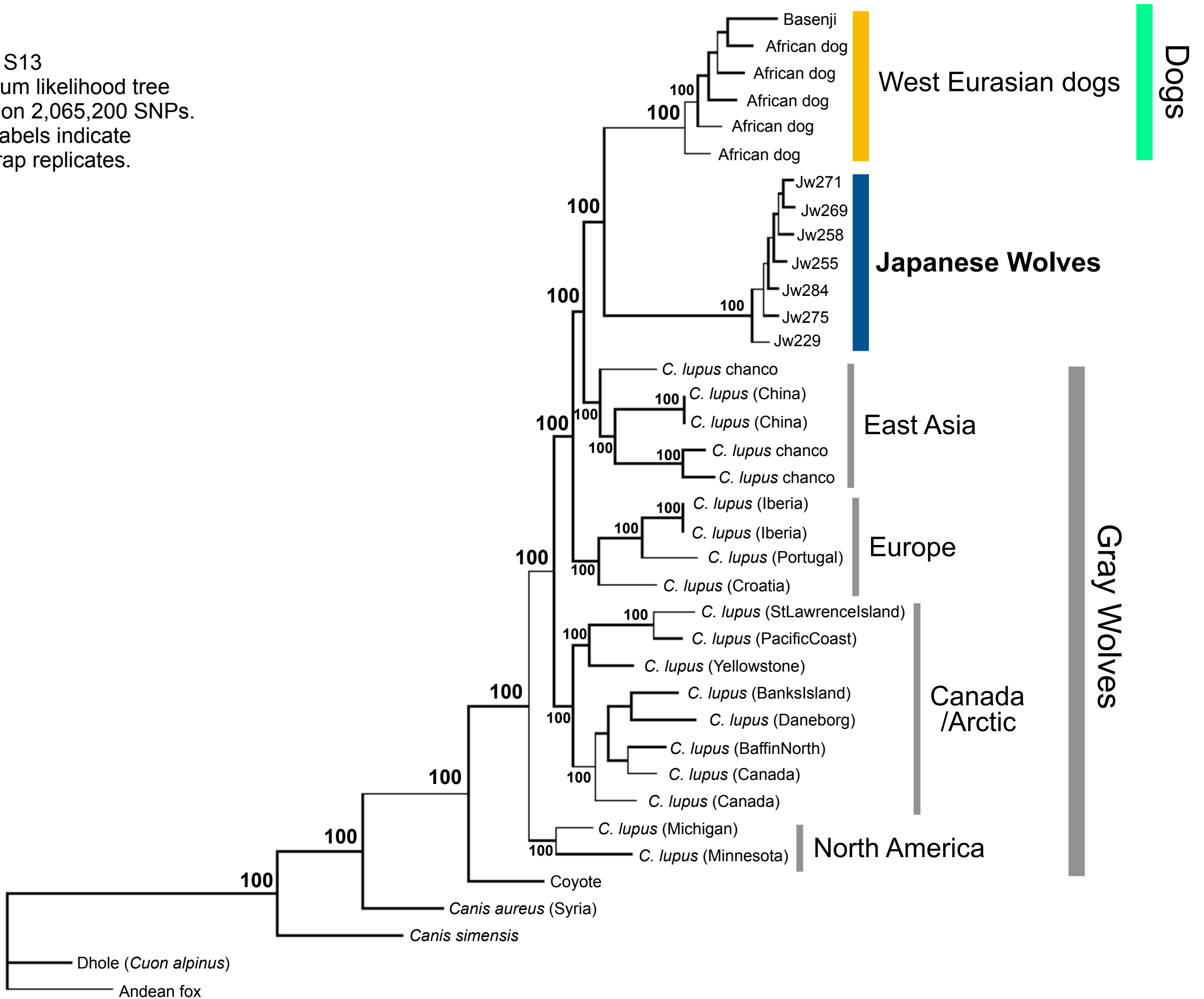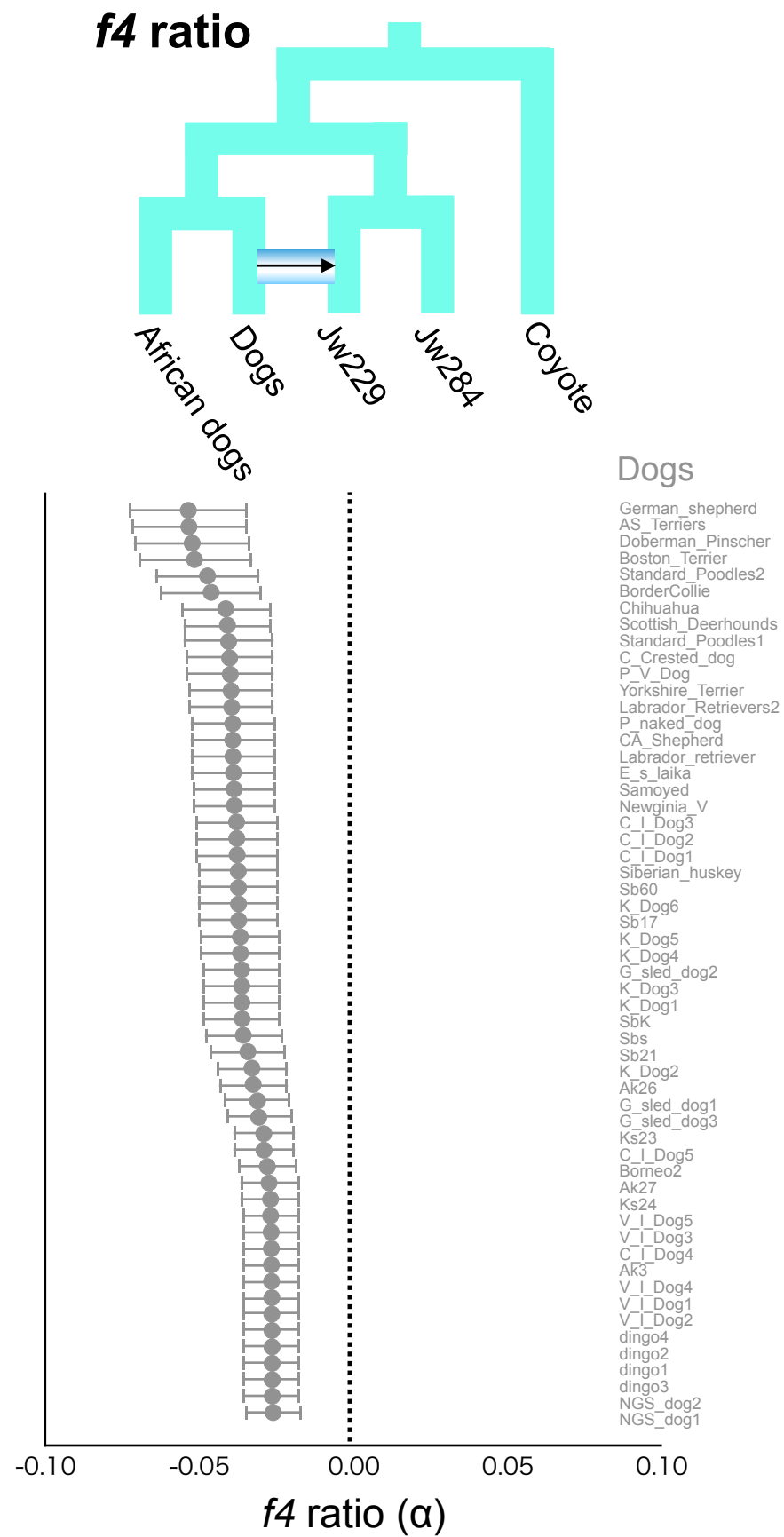
Figure S14

f4-ratio test to estimate proportion of genome introgression from dogs to the Japanese wolf. Each *f4*-ratio α value is plotted in order of lowest to highest value from the top, and the names of the dogs are shown on the right side of the panel. Error bars represent standard errors.
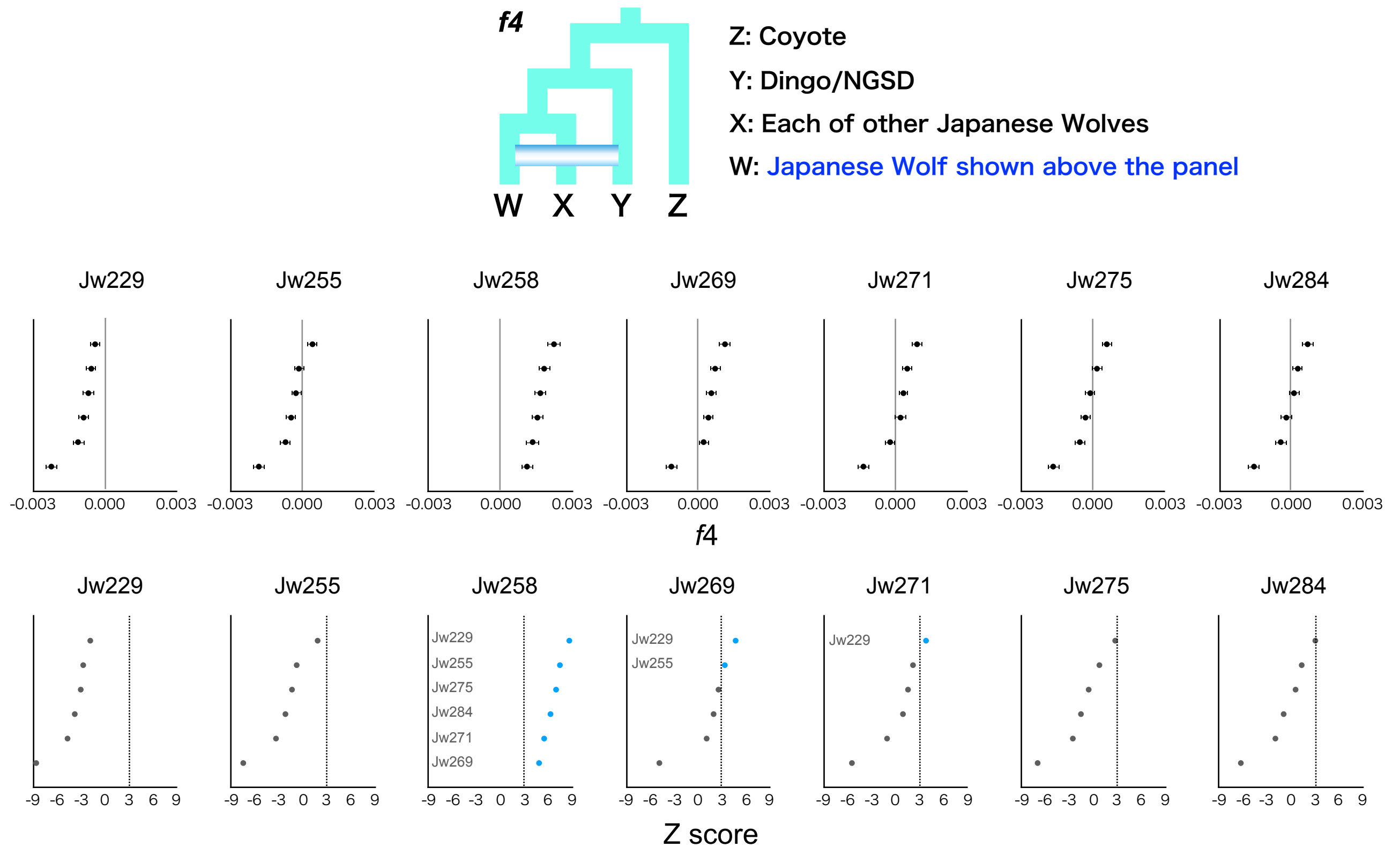
Figure S15

(A) *f4* statistics testing the difference of the affinity to dingo/NGSD between the Japanese wolf individuals. *f4* statistics (upper panels) and Z score (lower panels) value is plotted in order of highest to lowest value from the top. Z score above 3 is colored in blue. When the Japanese wolf individual showing a significant affinity to dingo/NGSD, the names of the Japanese wolf individual at the position X in the schematic representation are shown on the left side of Z score panel.
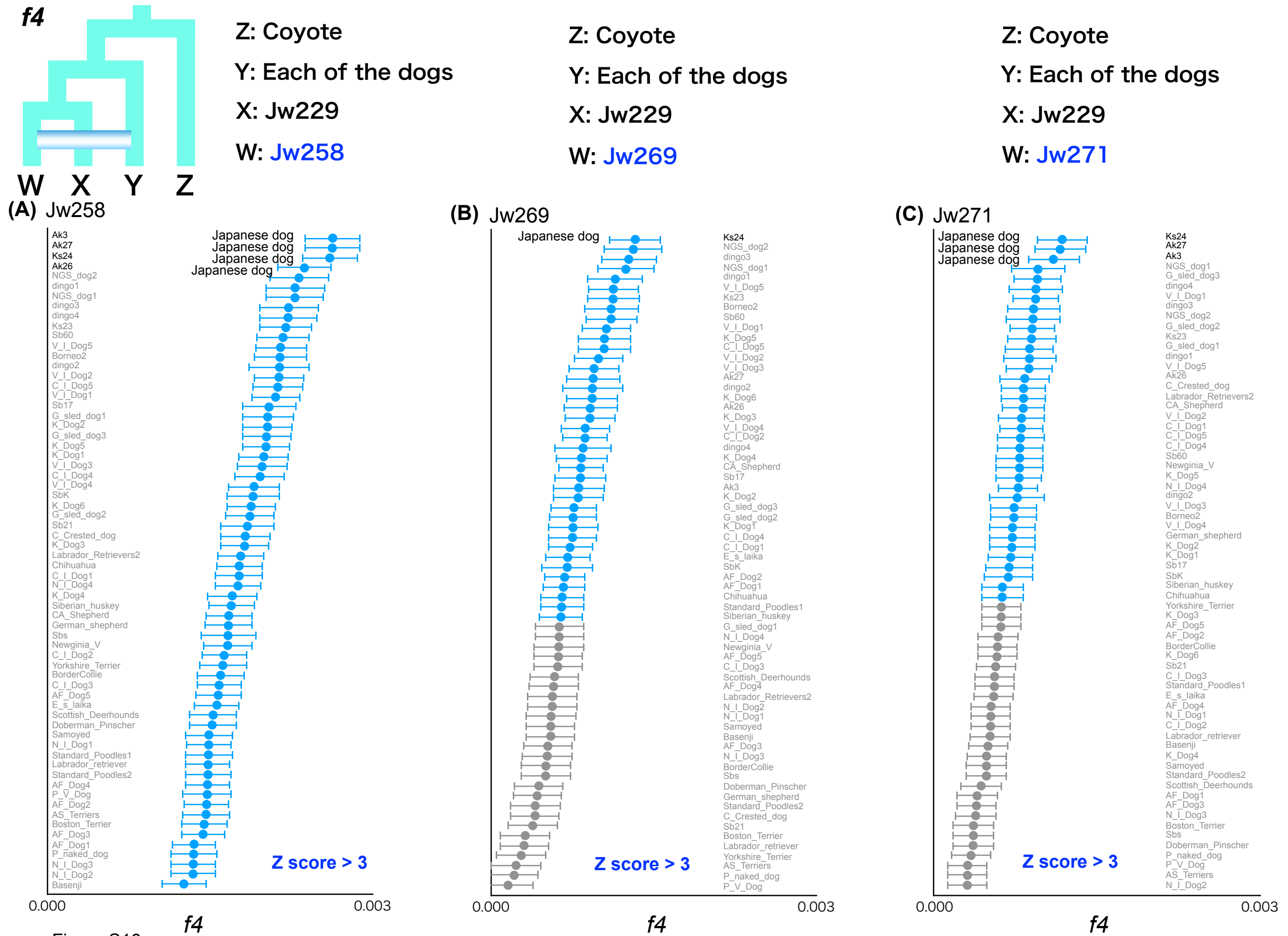
Figure S16

(A) *f4* statistics testing the relationships between (A) Jw258, (B) Jw269, and (C) Jw271 and all dog individuals. Each Z score is plotted in order of highest to lowest value from the top, and the names of dogs are shown on theft or right sides of each panel (see table S2). Z score above 3 is colored in blue.
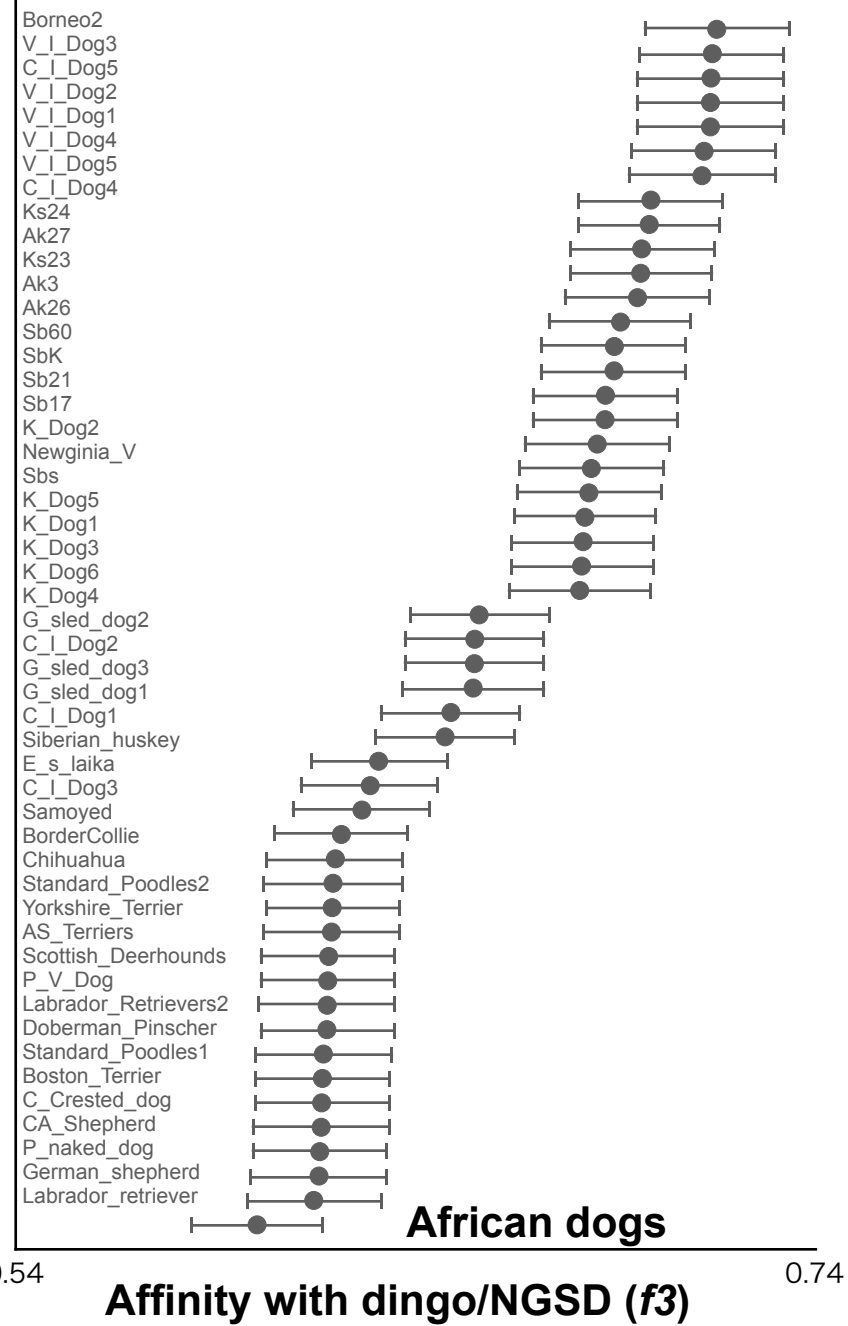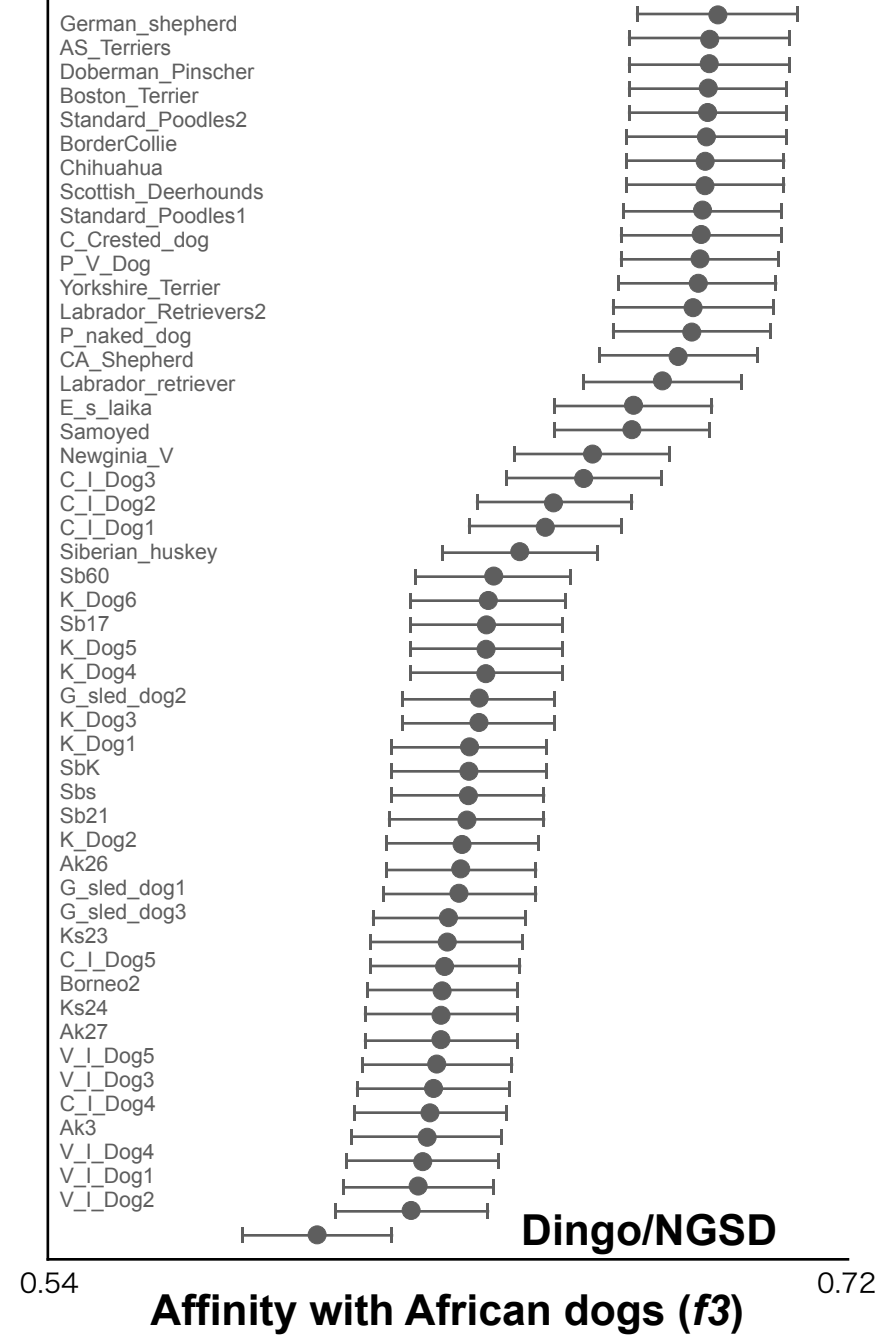
Figure S17
Shared genetic drift between (A) dingo/NGSD and (B) African dogs and all dogs measured by outgroup *f*3 statistics. Each of the African dogs and dingo/NGSD individuals were used as populations. Each *f*3 value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the left side of the panel. Error bars represent standard errors.
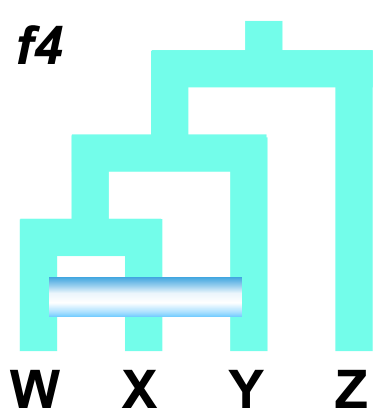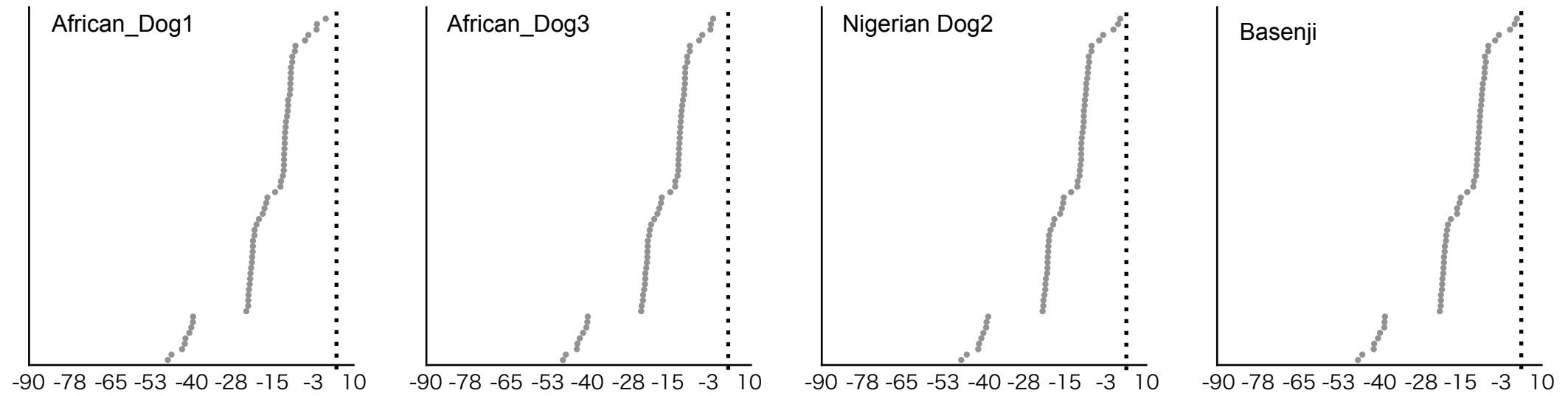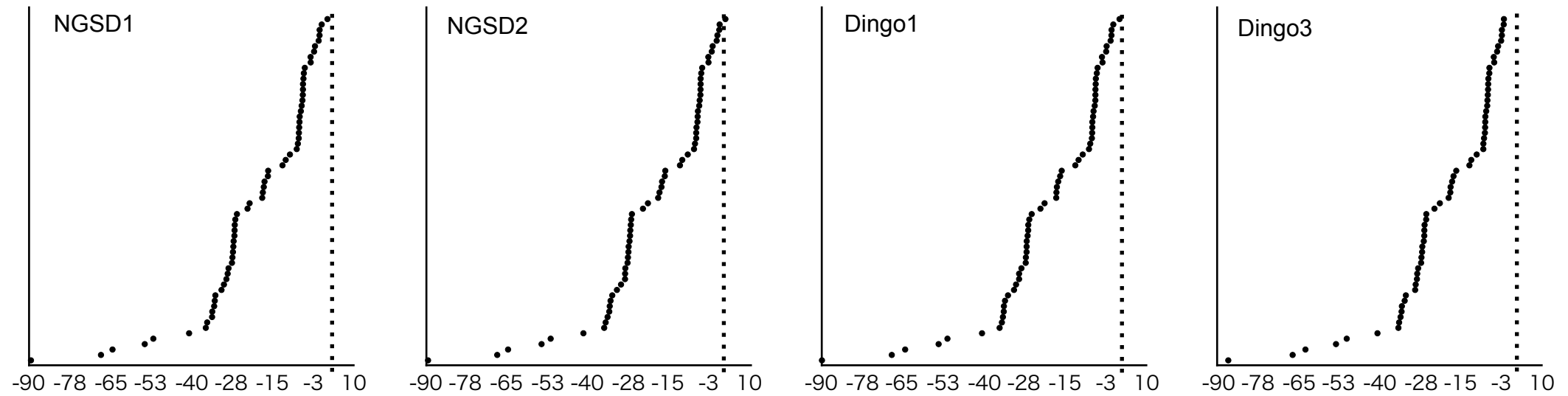
Figure S18

f4 statistics testing the affinity of NGSD1 with African dogs (upper panels) and that of Basenji with dingo/NGSD dogs (lower panels). All Z scores were under 3.

f4

Z: Coyote

Y: Dingo/NGSD

X: African dogs

W: Each of the dogs

Affinity with Dingo/NGSD (f4) (y-axis)

Sled dogs
Other dogs

Affinity with African dogs (f4) (x-axis)
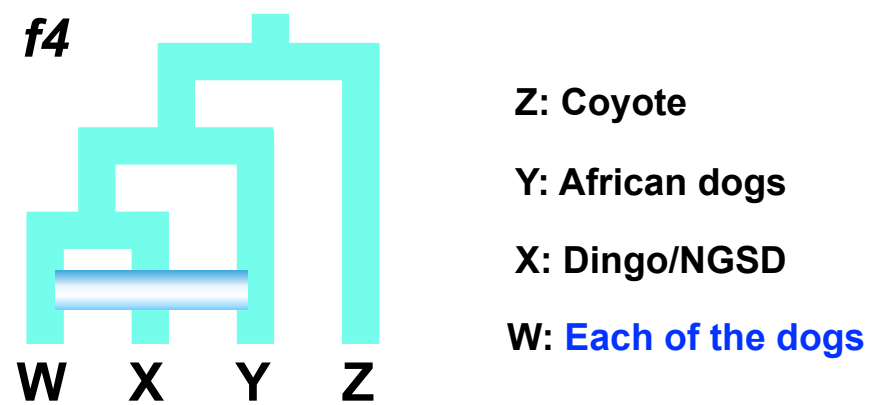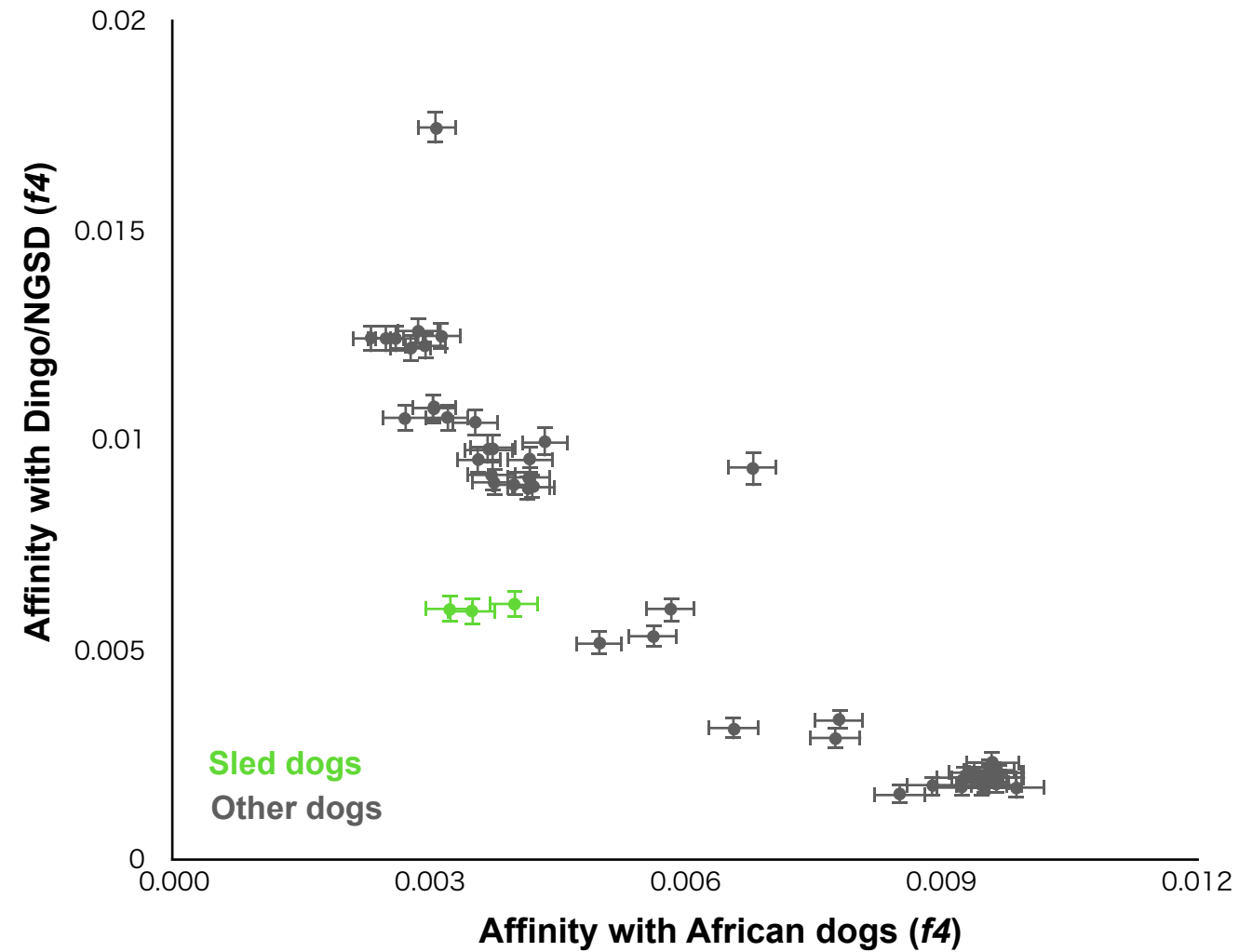
Figure S19
f4 statistics testing whether dogs share more alleles with African dogs (x-axis) or dingo/NGSD (y-axis) compared with dingo/NGSD and African dogs, respectively. Dots show the *f4* statistics, and horizontal and vertical error bars represent standard errors for the test with the African dogs (x-axis) and dingo/NGSD (y-axis), respectively. Each of the African dogs and dingo/NGSD individuals were used as populations.

f4

Z: Coyote

Y: African dogs

X: Dingo/NGSD

W: Each of the dogs

f3
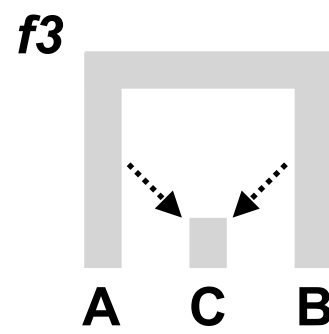
A: African dogs
B: Dingo/NGSD
C: Each of the dogs

A C B

Figure S20

f3 statistics testing the genomic mixture of African and Dingo/NGSD dogs in all dogs. Z score under -3 is colored in blue. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each *f3* value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the left side of the panel. Error bars represent standard errors.

Sbs
Doberman_Pinscher
Scottish_Deerhounds
P_V_Dog
German_shepherd
C_Crested_dog
Samoyed
G_sled_dog3
Sb60
G_sled_dog1
G_sled_dog2
BorderCollie
Labrador_Retrievers2
Standard_Poodles1
Ak3
AS_Terriers
Ak27
Siberian_huskey
Yorkshire_Terrier
Boston_Terrier
Standard_Poodles2
Ak26
Sb21
Chihuahua
Labrador_retriever
Borneo2
SbK
Sb17
C_I_Dog3
Ks24
Ks23
CA_Shepherd
P_naked_dog
K_Dog5
E_s_laika
K_Dog1
K_Dog6
C_I_Dog1
V_I_Dog2
C_I_Dog5
V_I_Dog5
V_I_Dog3
K_Dog4
V_I_Dog4
Newginia_V
C_I_Dog4
C_I_Dog2
K_Dog2
K_Dog3
V_I_Dog1

Z score < -3

-0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7

f3
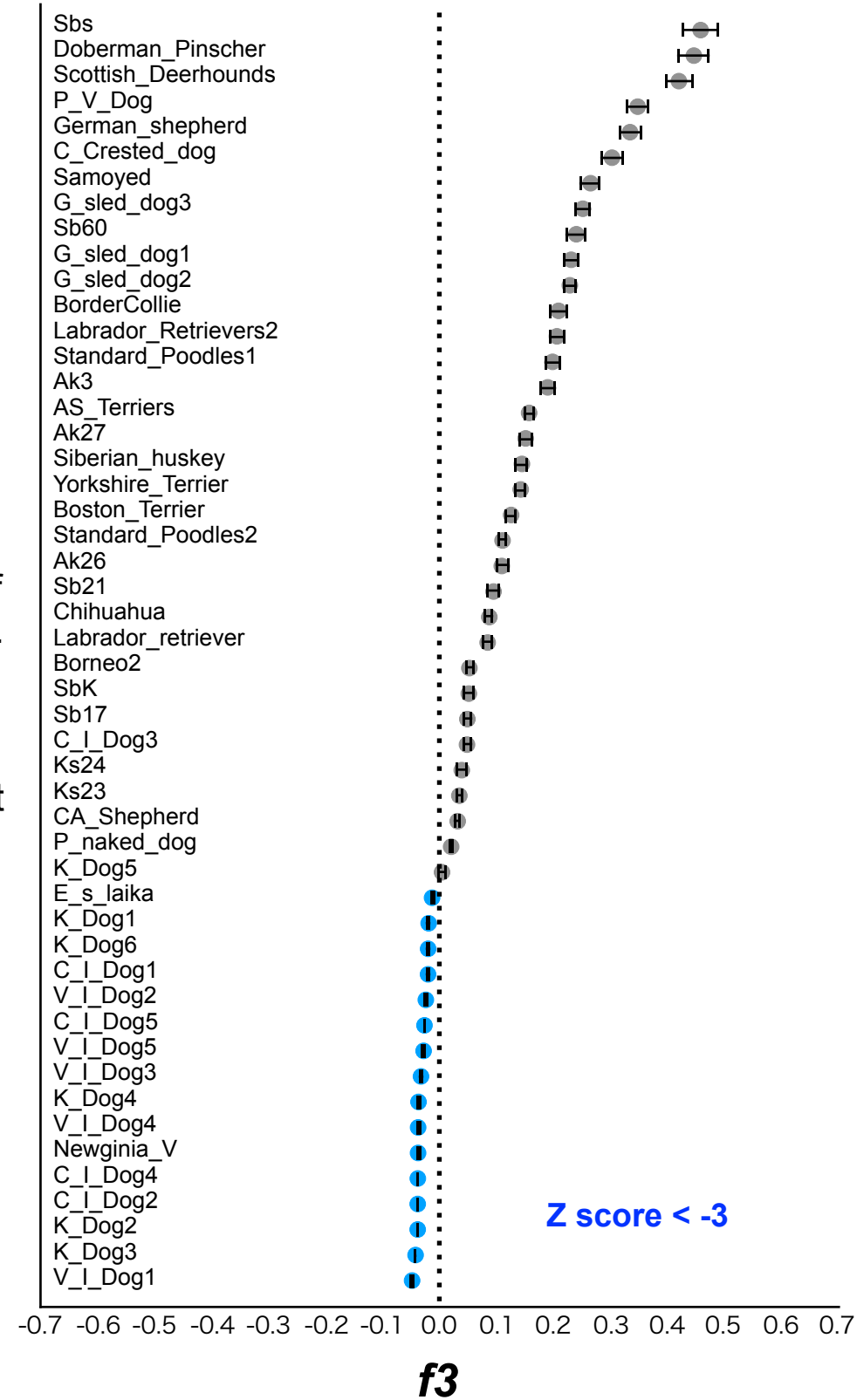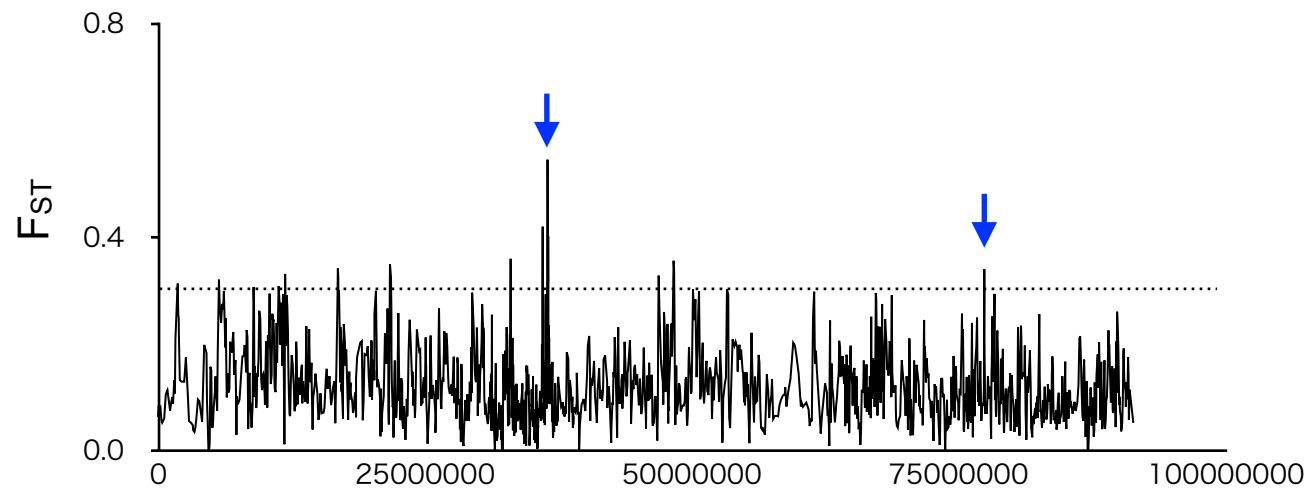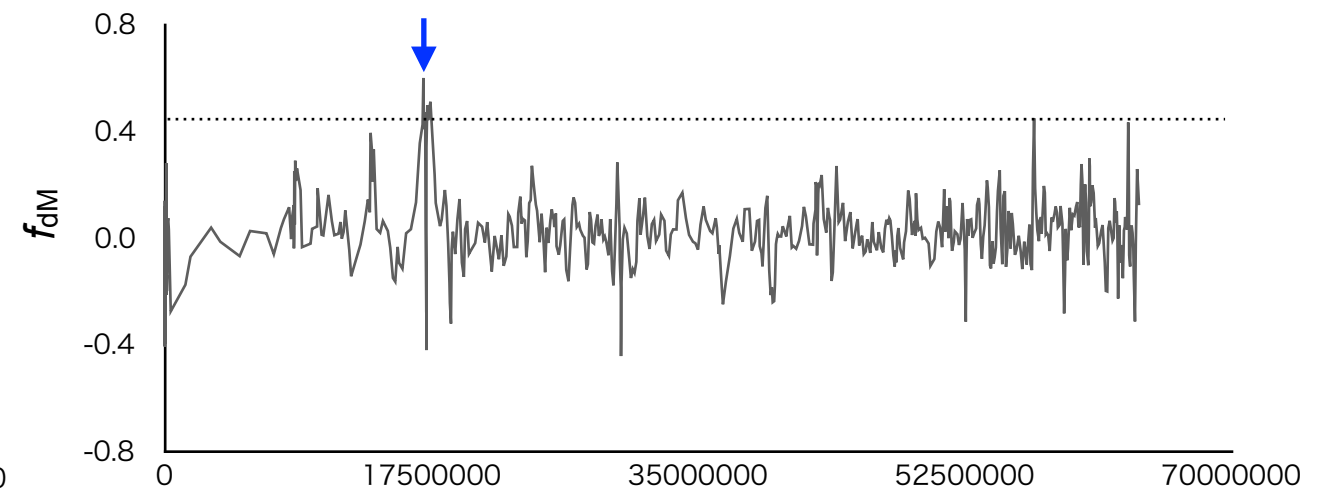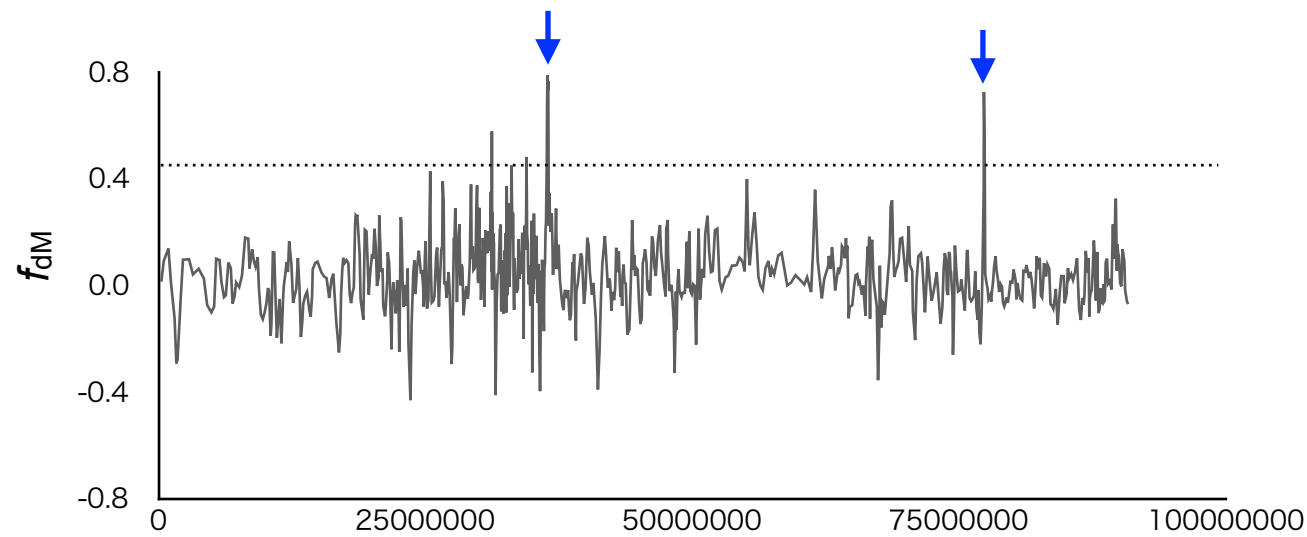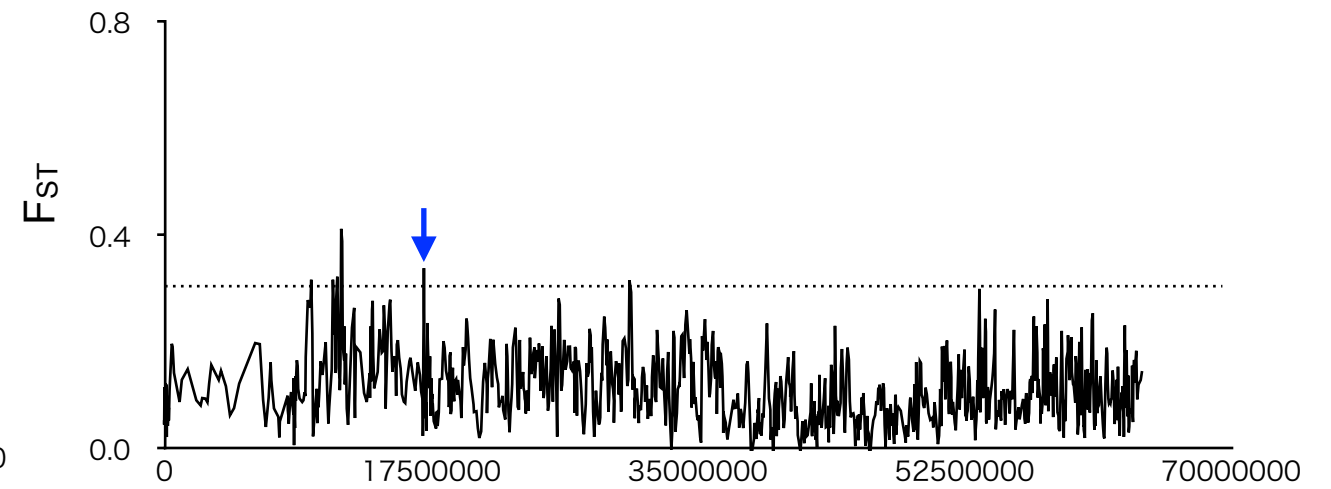
Figure S21
Sliding window analyses of the F_{ST} values (y-axis: upper panel) and f_{dM} (y-axis: lower panel) in windows of 50 SNPs using a 25 SNPs slide across scaffolds (x-axis). Dashed lines show the 99th percentiles. Blue arrow indicate overlapping regions above 99th percentiles between upper and lower panels.

Chromosome 19

Chromosome 30

Figure S21 continued
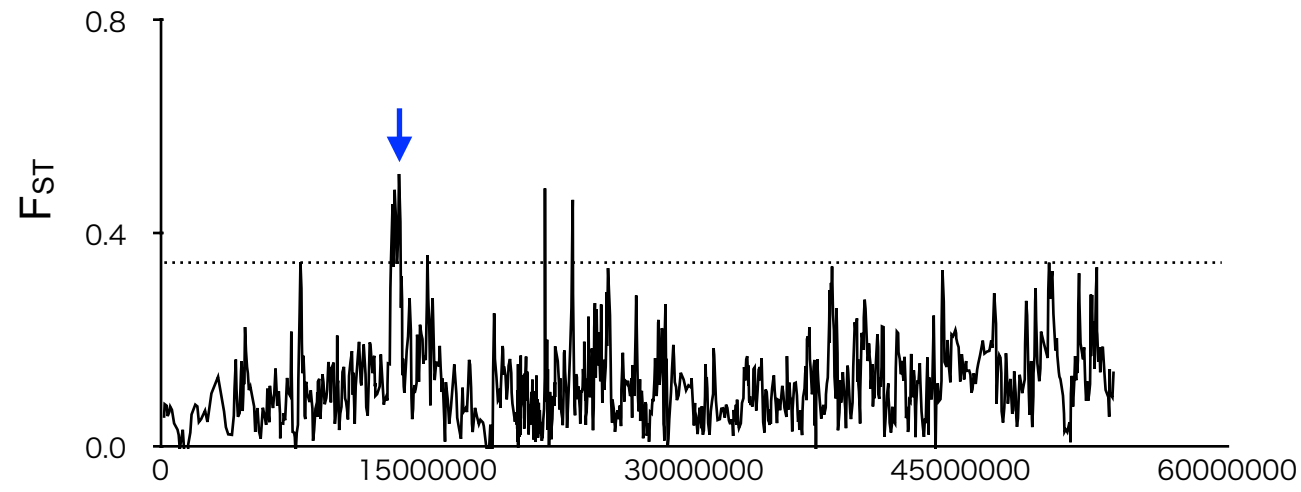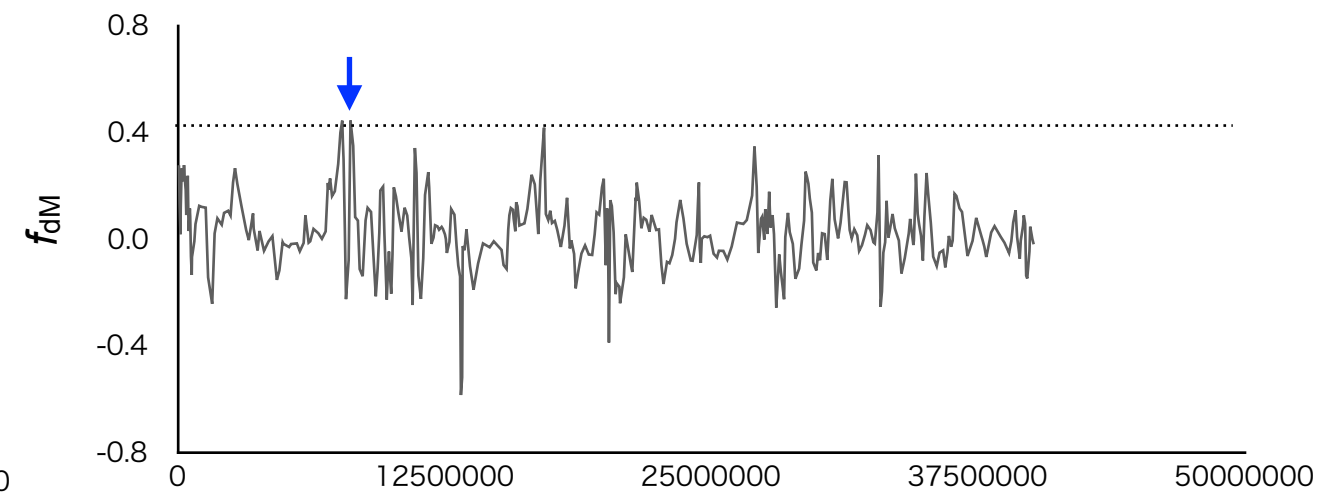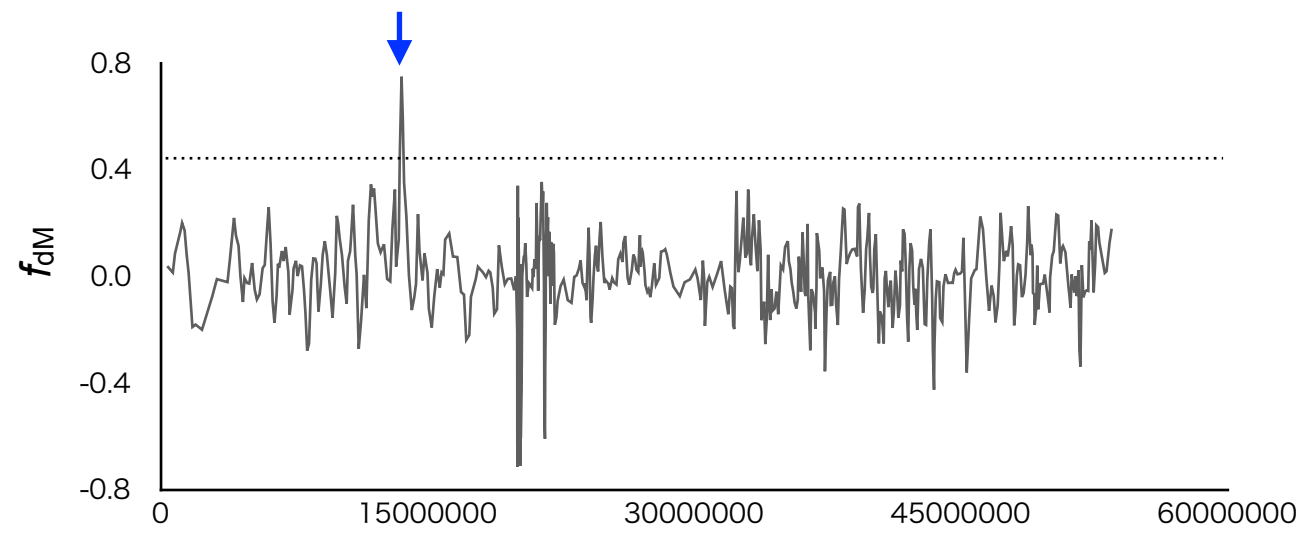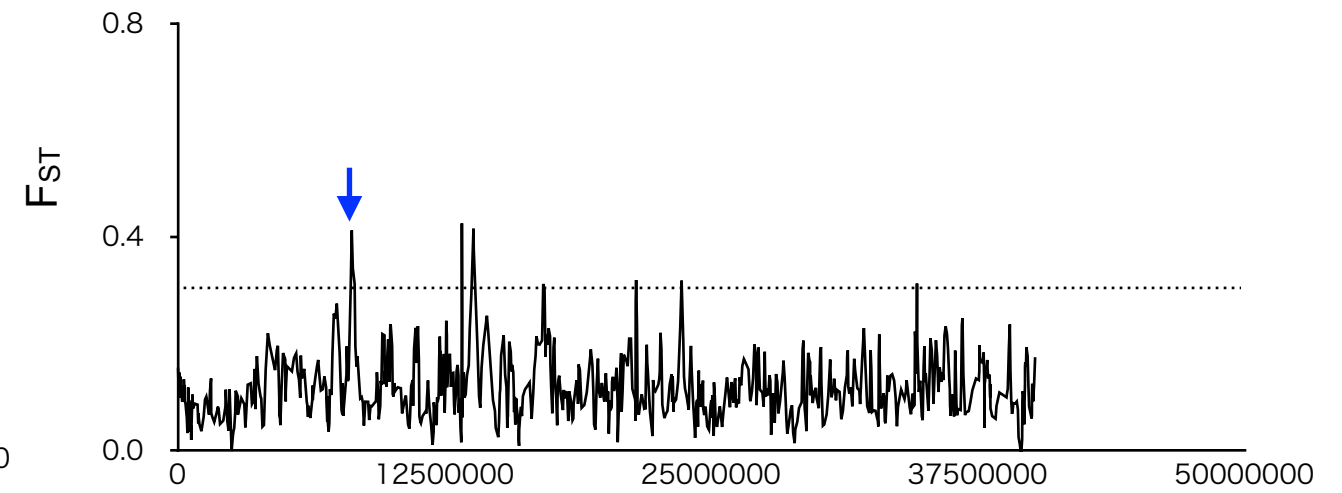
Table S1 Determined sequences in this study

Wolf/Dog	ID	Period	Isolation site (Prefecture)	Mapped reads count	Total mapped reads
Japanese Wolves	Leiden_b (Jentink 1887 b RMNH.MAM.39183)	Edo	Leiden	149 M	22.35 Gb
Japanese Wolves	Leiden_c (Jentink 1887 c RMNH.MAM.39181)	Edo	Leiden	188 M	28.2 Gb
Japanese Wolves	Jw255	Edo-Meiji	Yamanashi Pref.	1881 M	282.2 Gb
Japanese Wolves	Jw271	Edo-Meiji	Iwate Pref.	820 M	123 Gb
Japanese Wolves	Jw284 (ZMB_Mam_048817)	Meiji	Berlin	1237 M	185.6 Gb
Japanese Wolves	Jw275	Edo-Meiji	Shimane Pref.	134 M	46.5 Gb
Japanese Wolves	Jw229	Edo	Kochi Pref.	661 M	99.2 Gb
Japanese Wolves	Jw258	Edo-Meiji	Nagano Pref.	262 M	110 Gb
Japanese Wolves	Jw269	Edo-Meiji	Nagano Pref.	193 M	61.8 Gb
Dog (Akita)	Akita26	Modern	—	847 M	127.1 Gb
Dog (Akita)	Akita27	Modern	—	808 M	121.2 Gb
Dog (Akita)	Akita3	Modern	—	772 M	115.8 Gb
Dog (Kishu)	Kishu23	Modern	—	832 M	124.8 Gb
Dog (Kishu)	Kishu24	Modern	—	634 M	95.1 Gb
Dog (Shiba)	Shiba17	Modern	—	632 M	94.8 Gb
Dog (Shiba)	Shiba21	Modern	—	517 M	77.6 Gb
Dog (Shiba)	Shiba60	Modern	—	668 M	100.2 Gb
Dog (Shiba)	ShibaKuro	Modern	—	472 M	70.8 Gb
Dog (Shiba)	ShibaShiro	Modern	—	697 M	104.6 Gb
Dog (Shiba)	Jm	Modern	—	391 M	58 Gb

Table S2 Sample information

Accession#	Sub-species	Common name	IDs in analyses	PCA & ADMIXTURE (modern) — Without missing data	PCA with Pleistocene canid — Without missing data	PCA & ADMIXTURE with Leiden b, c and Honshu Wolf — With missing data	Phylogenetic analysis — Without missing data	f4 and f4-ratio with Leiden c — Without missing data	f4 with Leiden b — Without missing data	f3, f4, and f4-ratio — Without missing data	Fst sliding window — Without missing data	fdM sliding window — Without missing data	TreeMix — Without missing data
Depositing	Japanese Wolf	Japanese Wolf	Jw255	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw271	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw284	O	O	O	O	O	O	O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw275	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw229	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw258	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw269	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Leiden_b			O	O		O				
Depositing	-	Jama-inu	Leiden_c			O	O	O					
ERS5374233	Japanese Wolf	Honshu Wolf					O						
Depositing	Dog	Kishu	Ks23	O	O	O	O	O		O	O	O	O
Depositing	Dog	Kishu	Ks24	O	O	O	O	O		O	O	O	O
Depositing	Dog	Akita	Ak26	O	O	O	O	O		O	O	O	O
Depositing	Dog	Akita	Ak27	O	O	O	O	O		O	O	O	O
Depositing	Dog	Akita	Ak3	O	O	O	O	O		O	O	O	O
Depositing	Dog	Shiba	Sb17	O	O	O	O	O		O	O	O	O
Depositing	Dog	Shiba	Sb21	O	O	O	O	O		O	O	O	O
Depositing	Dog	Shiba	Sb60	O	O	O	O	O		O	O	O	O
Depositing	Dog	Shiba	SbK	O	O	O	O	O		O	O	O	O
Depositing	Dog	Shiba	Sbs	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Shiba	Jm	O	O	O	O					O	O
SRR10596312	Dog	Dingo	dingo1	O	O	O	O	O		O			O
SRR10596315	Dog	Dingo	dingo2	O	O	O	O	O		O			O
SRR10596318	Dog	Dingo	dingo3	O	O	O	O	O		O			O
SRR10596321_1	Dog	Dingo	dingo4	O	O	O	O	O		O			O
SRR10597858	Dog	New Guinea Singing Dog	NGS_dog1	O	O	O	O	O		O			O
SRR10597859	Dog	New Guinea Singing Dog	NGS_dog2	O	O	O	O	O		O			O
SRR2827578_1	Dog	Chinese Indigenous Dog	C_I_Dog1	O	O	O	O	O		O			
SRR2827583_1	Dog	Chinese Indigenous Dog	C_I_Dog2	O	O	O	O	O		O			
SRR2827587_1	Dog	Chinese Indigenous Dog	C_I_Dog3	O	O	O	O	O		O			
SRR2827588_1	Dog	Chinese Indigenous Dog	C_I_Dog4	O	O	O	O	O		O			
SRR2827589_1	Dog	Chinese Indigenous Dog	C_I_Dog5	O	O	O	O	O		O			
SRR2827595_1	Dog	Vietnamese Indigenous Dog	V_I_Dog1	O	O	O	O	O		O			O
SRR2827596_1	Dog	Vietnamese Indigenous Dog	V_I_Dog2	O	O	O	O	O		O			O
SRR2827597_1	Dog	Vietnamese Indigenous Dog	V_I_Dog3	O	O	O	O	O		O			O
SRR2827598_1	Dog	Vietnamese Indigenous Dog	V_I_Dog4	O	O	O	O	O		O			O
SRR2827599_1	Dog	Vietnamese Indigenous Dog	V_I_Dog5	O	O	O	O	O		O			O
SRR5177184_1	Dog	DongGyeongi	K_Dog1	O	O	O	O	O		O			O
SRR5177186_1	Dog	DongGyeongi	K_Dog2	O	O	O	O	O		O			O
SRR5177187_1	Dog	DongGyeongi	K_Dog3	O	O	O	O	O		O			O
SRR5177188_1	Dog	DongGyeongi	K_Dog4	O	O	O	O	O		O			O
SRR5177189_1	Dog	DongGyeongi	K_Dog5	O	O	O	O	O		O			O
SRR5177190_1	Dog	DongGyeongi	K_Dog6	O	O	O	O	O		O			O
SRR11193488_1	Dog	Greenland sled dog	G_sled_dog1	O	O	O	O	O		O			O
SRR11193493_1	Dog	Greenland sled dog	G_sled_dog2	O	O	O	O	O		O			O
SRR11193499_1	Dog	Greenland sled dog	G_sled_dog3	O	O	O	O	O		O			O
SRR2827574_1	Dog	Siberian huskey	Siberian_huskey	O	O	O	O	O		O			O
SRR2827565_1	Dog	East siberian laika	E_s_laika	O	O	O	O	O		O			O
SRR2827573_1	Dog	Samoyed	Samoyed	O	O	O	O	O		O			O
SRR5381785_1	Dog	Central Asian Shepherd	CA_Shepherd	O	O	O	O	O		O			O
SRR1594157_1	Dog	Chinese Crested dog	C_Crested_dog	O	O	O	O	O		O			O
SRR2827564_1	Dog	Chihuahua	Chihuahua	O	O	O	O	O		O			O
SRR2827572_1	Dog	Peruvian naked dog	P_naked_dog	O	O	O	O	O		O			
SRR3384031_1	Dog	Portugal Village Dog	P_V_Dog	O	O	O	O	O		O			O
SRR3466766_1	Dog	BorderCollie	BorderCollie	O	O	O	O	O		O			O
SRR6297982_1	Dog	Doberman Pinscher	Doberman Pinscher	O	O	O	O	O		O			O
SRR6376929_1	Dog	American Staffordshire Terriers	AS_Terriers	O	O	O	O	O		O			O
SRR7107687	Dog	Borneo Village Dog	Borneo2	O	O	O	O	O		O			O
SRR10208218_1	Dog	Boston Terrier	Boston Terrier	O	O	O	O	O		O			O
SRR10250959_1	Dog	Scottish Deerhounds	Scottish Deerhounds	O	O	O	O	O		O			O
SRR9117685_1	Dog	German shepherd	German shepherd	O	O	O	O	O		O			O
SRR11787131_1	Dog	Labrador retriever	Labrador retriever	O	O	O	O	O		O			O
SRR9962250_1	Dog	Labrador Retriever	Labrador_Retrievers2	O	O	O	O	O		O			O
SRR7107697	Dog	Newginia Village Dog	Newginia_V	O	O	O	O	O		O			O
SRR7764562_1	Dog	Yorkshire Terrier	Yorkshire Terrier	O	O	O	O	O		O			O
SRR8163589_1	Dog	Standard Poodle	Standard_Poodles1	O	O	O	O	O		O			O
SRR8163591_1	Dog	Standard Poodle	Standard_Poodles2	O	O	O	O	O		O			O
SRR2827591_1	Dog	Nigerian Indigenous Dog	N_I_Dog1	O	O	O	O	O		O	O	O	O
SRR2827592_1	Dog	Nigerian Indigenous Dog	N_I_Dog2	O	O	O	O	O		O	O	O	O
SRR2827593_1	Dog	Nigerian Indigenous Dog	N_I_Dog3	O	O	O	O	O		O			O
SRR2827594_1	Dog	Nigerian Indigenous Dog	N_I_Dog4	O	O	O	O	O		O			O
SRR2149861_1	Dog	Basenji	Basenji	O	O	O	O	O		O			O
SRR3603090_1	Dog	African dogs	AF_Dog1	O	O	O	O	O		O	O	O	O
SRR3603171_1	Dog	African dogs	AF_Dog2	O	O	O	O	O		O	O	O	O
SRR3603205_1	Dog	African dogs	AF_Dog3	O	O	O	O	O		O	O	O	O
SRR3603206_1	Dog	African dogs	AF_Dog4	O	O	O	O	O		O	O	O	O
SRR3603236_1	Dog	African dogs	AF_Dog5	O	O	O	O	O		O	O	O	O
SRR2017901_1	Gray Wolf	Himalayan Wolf	Wolf_chanco1	O	O	O	O			O			O
SRR2017902_1	Gray Wolf	Himalayan Wolf	Wolf_chanco2	O	O	O	O			O			O
SRR2017904_1	Gray Wolf	Himalayan Wolf	Wolf_chanco3	O	O	O	O			O			O
SRR1518523_1	Gray Wolf	Gray Wolf (Portugal)	Wolf Portugal	O	O	O	O			O			
SRR2149870_1	Gray Wolf	Israeli Wolf	Wolf_Israel	O	O	O	O			O			
SRR2149873_1	Gray Wolf	Croatian Wolf	Wolf_Croatian	O	O	O	O			O			O
SRR2149879_1	Gray Wolf	Chinese Wolf	Wolf_Chinese1	O	O	O	O			O			O
SRR2149880_1	Gray Wolf	Chinese Wolf	Wolf_Chinese2	O	O	O	O			O			O
SRR3384029_1	Gray Wolf	Iberian Wolf	Wolf_Iberia1	O	O	O	O			O			O
SRR3384030_1	Gray Wolf	Iberian Wolf	Wolf_Iberia2	O	O	O	O			O			O
SRR3574809	Gray Wolf	Indian Wolf	Wolf_India	O	O	O	O			O			
SRR8049193_1	Gray Wolf	Grey Wolf (Saudi Arabia)	Wolf_Saudi_Arabia	O	O	O	O			O			
SRR8049194_1	Gray Wolf	Grey Wolf (Syria)	Wolf_Syria	O	O	O	O			O			
SRR8049195_1	Gray Wolf	Grey Wolf (Daneborg)	Wolf_Daneborg	O	O	O	O			O			
SRR8066603_1	Gray Wolf	Grey Wolf (StLawrenceIsland)	Wolf_StLawrenceIsland	O	O	O	O			O			
SRR8066604_1	Gray Wolf	Grey Wolf (PacificCoast)	Wolf_PacificCoast	O	O	O	O			O			
SRR8066607_1	Gray Wolf	Grey Wolf (BaffinNorth)	Wolf_BaffinNorth	O	O	O	O			O			
SRR8066615_1	Gray Wolf	Grey Wolf (BanksIsland)	Wolf_BanksIsland	O	O	O	O			O			
SRR8380700_1	Gray Wolf	Grey Wolf (Michigan)	Wolf_Michigan	O	O	O	O			O			
SRR8380701_1	Gray Wolf	Grey Wolf (Minnesota)	Wolf_Minnesota	O	O	O	O			O			
SRR8380706_1	Gray Wolf	Grey Wolf (Canada)	Wolf_Canad1	O	O	O	O			O			
SRR8380708_1	Gray Wolf	Grey Wolf (Canada)	Wolf_Canad2	O	O	O	O			O			
SRR9095639_1	Gray Wolf	Grey Wolf (Yellowstone)	Wolf_Yellowstone	O	O	O	O			O			
SRR3417116	Ancient dog	Ancient dog	Cherry Tree Cave Dog		O								
SRR3417117	Ancient dog	Ancient dog	Herxheim Dog		O								
SRR11193507	Ancient dog	Ancient dog	Zhokhov Dog		O								
ERR1341514	Ancient dog	Ancient dog	Newgrange Dog		O								
ERR4805960	Pleistocene canid	Pleistocene canid	Bunge-Toll-1885		O								
ERR4805984	Pleistocene canid	Pleistocene canid	Tirekhtyakh		O								
ERR4805988	Pleistocene canid	Pleistocene canid	Tumat 2		O								
ERR4805972	Pleistocene canid	Pleistocene canid	Ulakhan Sular		O								
SRR11193495	Pleistocene canid	Pleistocene canid	Yana RHS		O								
SRR7107770	out group	Coyote	Coyote	O	O	O	O	O		O			O
SRR8049189	out group	Dhole	Dhole				O						
SRR8049190	out group	Ethiopian wolf	Ethi_W				O						
SRR8049192	out group	Golden Jackal (Syria)	G_Jackal_Syria	O	O	O	O						
PRJCA000335	out group	African Golden Wolf	AGW	O	O	O	O						
SRR1066702	out group	Andean fox	ad_fox				O						

f4 ratio

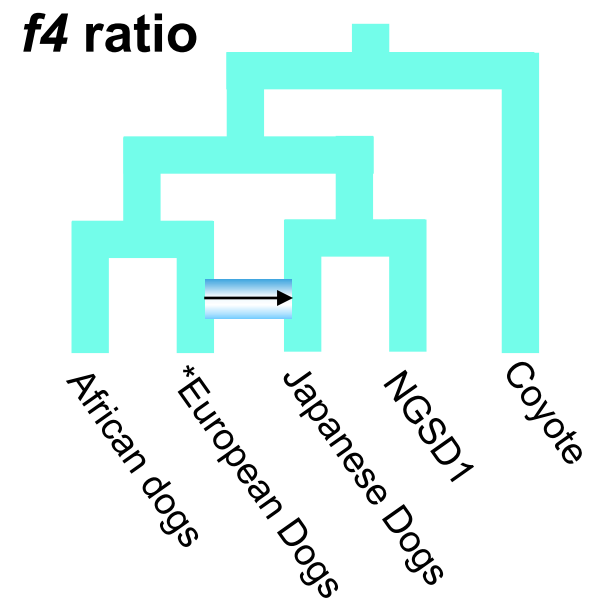Tree tips (left to right): African dogs, *European Dogs, Japanese Dogs, NGSD1, Coyote

Table S3 The degree of mixing of the European dog genome into the Japanese dog genome

Japanese Dogs	*f4*-ratio (alpha)	Standard error	Z score
Akita	**0.363**	0.019328	18.768
Ks	**0.366**	0.020174	18.133
Shiba	**0.445**	0.017633	25.237

*European Dogs: Yorkshire_Terrier, P_V_Dog, Standard_Poodles1, Standard_Poodles2, Scottish_Deerhounds, BorderCollie, Boston_Terrier, Doberman_Pinscher, AS_Terriers, German_shepherd.
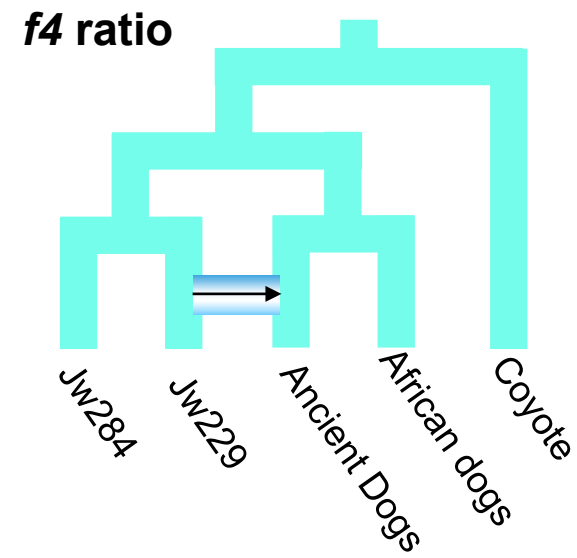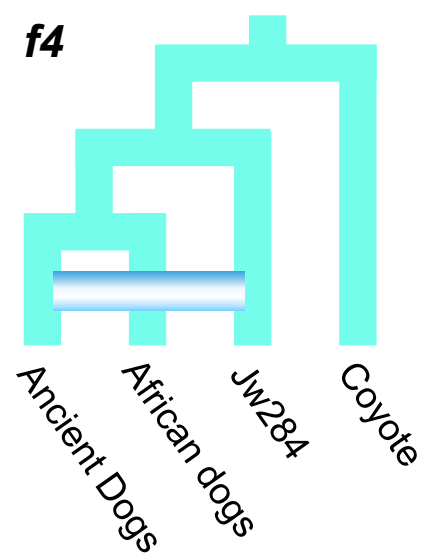See Table S2 for the name of the dogs.

Table S4 Gene flow between Japanese Wolf and ancient dogs

Ancient Dogs	Years ago	f4	stderr	Zscore	f4-ratio(alpha)	stderr	Zscore
Newgrange	4800	0.003171	0.000614	*5.163*	0.015764	0.00305	*5.168*
Cherry Tree Cave	5000	0.001028	0.000568	1.81	0.005231	0.002874	1.82
Herxheim	7000	0.003199	0.000606	*5.278*	0.016101	0.003041	*5.295*
Zhokhov	9500	0.004214	0.000715	*5.897*	0.021478	0.003612	*5.947*

Table S5 Genomic regions differentiated from Western Eurasian dogs and derived from Japanese Wolf

Chromosome	5' end	3' end	Genes in the region
2	6257975	6307631	*Ankyrin repeat domain 26 (ANKRD26)
3	36520617	36555078	Upstream of MKRN3, Downstream of CHRNA7
3	77467500	77472016	-
15	17047771	17058407	Upstream of FAM183A
19	13299716	13485166	**Upstream of INTU
30	8023335	8222693	INO80, ***DLL4, VPS18

*Mice mutant in one of these genes, ANKRD26, become hyperphagia and also have enhanced adipocyte differentiation (Bera, et al. 2008; Fei, et al. 2011). The traits resulting from the mutation of this gene may have been advantageous to the diet in the Japanese archipelago during Japanese dog evolution.

**INTU is part of a protein network called CPLANE, and mutations in INTU cause a number of skeletal morphological abnormalities, one of which is a flat nasal bridge (Toriyama, et al. 2016). Japanese wolves and Japanese dogs of the Jomon period (still found in a small number of Japanese dogs today) are known to have shallow stops, and perhaps mutations in this gene are related to the formation of shallow stops.

***Notch signaling plays an important role in the development and differentiation of many cell types in diverse organisms, and DLL4, the ligand for the Notch receptor, is known to have many roles during development (Benedito and Duarte 2005). Although this gene is multifunctional and therefore traits could not be identified, it may be involved in shaping the characteristics of Japanese dogs through the Notch signaling pathway.

Reference
- Benedito R, Duarte A. 2005. Expression of Dll4 during mouse embryogenesis suggests multiple developmental roles. Gene expression patterns 5:750-755.
- Bera TK, Liu X-F, Yamada M, Gavrilova O, Mezey E, Tessarollo L, Anver M, Hahn Y, Lee B, Pastan I. 2008. A model for obesity and gigantism due to disruption of the Ankrd26 gene. Proceedings of the National Academy of Sciences 105:270-275.
- Fei Z, Bera TK, Liu X, Xiang L, Pastan I. 2011. Ankrd26 gene disruption enhances adipogenesis of mouse embryonic fibroblasts. Journal of Biological Chemistry 286:27761-27768.
- Toriyama M, Lee C, Taylor SP, Duran I, Cohn DH, Bruel A-L, Tabler JM, Drew K, Kelly MR, Kim S. 2016. The ciliopathy-associated CPLANE proteins direct basal body recruitment of intraflagellar transport machinery. Nature genetics 48:648-656.

Table S6 Mapping rate (%) of reads with Japanese Wolf specific substitutions in the mitochondria DNA

Position in CanFam3.1	2730	3346	4400	4766	5828	6461	7732	8473	9514	10464	10701	11461	12062	15225	15490	Mean (%)	Estimated maximum contamination (%)
Jw258	97.0	97.0	94.9	96.3	99.8	100.0	100.0	99.8	100.0	100.0	100.0	100.0	96.4	99.8	100.0	98.7	1.3
Jw269	70.3	80.3	75.0	76.7	86.7	100.0	97.9	83.8	84.2	89.3	91.0	84.9	81.8	86.0	75.0	84.2	15.8
Jw229	92.5	93.9	94.8	94.4	97.2	99.7	97.0	96.3	95.9	97.8	97.6	97.0	90.9	95.2	95.8	95.7	4.3
Jw255	95.3	95.9	95.3	95.4	97.9	99.9	98.8	98.3	97.8	98.1	98.1	98.5	96.8	98.0	97.9	97.5	2.5
Jw284	99.6	99.6	99.7	99.7	99.9	99.8	99.8	99.8	99.7	99.8	99.9	99.9	99.7	99.8	99.7	99.8	0.2
Jw271	98.5	98.7	98.5	98.6	99.2	99.9	99.4	99.2	99.1	99.0	99.2	99.1	98.9	99.5	98.3	99.0	1.0
Jw275	98.6	98.4	98.1	98.0	99.3	100.0	99.5	99.5	99.0	99.0	98.6	98.6	97.7	99.0	99.0	98.8	1.2
Leiden_c	100.0	98.8	95.6	100.0	99.2	100.0	99.3	100.0	99.2	99.0	100.0	100.0	98.9	100.0	100.0	99.3	0.7
Leiden_b	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	100.0	99.9	95.7	100.0	94.5	98.8	100.0	99.3	0.7