Single Nucleotide Polymorphism (SNP) Variation of Wolves (*Canis lupus*) in Southeast Alaska and Comparison with Wolves, Dogs, and Coyotes in North America

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Abstract

There is considerable interest in the genetics of wolves (*Canis lupus*) because of their close relationship to domestic dogs (*C. familiaris*) and the need for informed conservation and management. This includes wolf populations in Southeast Alaska for which we determined genotypes of 305 wolves at 173 662 single nucleotide polymorphism (SNP) loci. After removal of invariant and linked SNP, 123 801 SNP were used to quantify genetic differentiation of wolves in Southeast Alaska and wolves, coyotes (*C. latrans*), and dogs from other areas in North America. There is differentiation of SNP allele frequencies between the species (wolves, coyotes, and dogs), although differentiation is relatively low between some wolf and coyote populations. There are varying levels of differentiation among populations of wolves, including low differentiation of wolves in interior Alaska, British Columbia, and the northern US Rocky Mountains. There is considerable differentiation of SNP allele frequencies of wolves in Southeast Alaska from wolves in other areas. However, wolves in Southeast Alaska are not a genetically homogeneous group and there are comparable levels of genetic differentiation among areas within Southeast Alaska and between Southeast Alaska and other geographic areas. SNP variation and other genetic data are discussed regarding taxonomy and management.

Subject areas: *Population structure and phylogeography, Conservation genetics and biodiversity*
Key words: *coyote, dog, genetic variation, single nucleotide polymorphism, SNP, wolf*

Understanding the genetics of the wolf (*Canis lupus*) is important because of its close relationship to the domestic dog (*C. familiaris*) and the application of genetic approaches to wildlife conservation and management. Dogs were domesticated from wolf ancestors as recently as 13 000–17 000 years ago (based on archaeological evidence) to more than 100 000 years ago (based on molecular clock estimates, Vilà et al. 1997, Honeycutt 2010). The genetic variation inherent in wolf progenitors permitted concerted selection and the development of more than 400 dog breeds that display great phenotypic divergence. Comparative studies of the genetic underpinnings of wolves and dogs can help understanding the basis of phenotypic variation, genealogy, and phylogeny of both species (Honeycutt 2010; vonHoldt et al., 2010, 2011; Brown et al. 2011; Vaysse et al., 2011; Wayne and vonHoldt 2012).

In this article, we describe the genetic variation of wolves and compare it with that of dogs and of coyotes (*C. latrans*), the latter which diverged from the wolf lineage approximately 1–2 million years ago (Nowak 1979; Kurtén and Anderson 1980). This is an important topic because of the uncertain systematic relationships and taxonomy of canid taxa in North America (Chambers et al. 2012). Recent

assessment of wolf genetic profiles have been used for both basic science (genomics, systematics, taxonomy, and population genetics, e.g., Vilà et al. 1999; Wayne and von-Holdt 2012) as well as applied management (e.g., identification of potential inbreeding, hybridization, and population management units, e.g., Carmichael et al. 2008; Hedrick and Fredrickson 2008; Monzón et al. 2014). Our study contributes to the basic science of North American canid systematics and taxonomy and also has relevance to conservation and management.

The emergence of novel genetic methods utilizing large numbers of genetic markers (single nucleotide polymorphisms SNP) allows greater resolution of relationships and ancestry of populations than previous methods. For example, vonHoldt et al. (2011) clarified taxonomy by applying SNP data to determine that extant wolves in the US Great Lakes region (considered *C. l. lycaon* or *C. lycaon*) and the red wolf (considered *C. l. rufus* or *C. rufus*) have admixed wolf and coyote ancestry. The taxonomy of wolves in other regions is also uncertain, including Southeast Alaska and coastal British Columbia. Wolf populations in this region are relatively intact compared to other areas of North America in which wolves were exterminated or greatly reduced in numbers. Morphological analyses suggested the wolves in Southeast Alaska were a distinct subspecies (*C. l. ligoni*, Hall 1981) but reanalysis combined these with a subspecies (*C. l. nubilus*) that occurs across much of North America (Figure 1, Nowak 1995, 2002). However, *C. l. ligoni* is still used by some authors (Weckworth et al. 2005, 2010, 2011; MacDonald and Cook 2009) and has

been proposed as an endangered subspecies in Southeast Alaska (CBD 2011; USFWS 2014).

Genetic studies have shown that wolves in southeast Alaska are genetically differentiated from wolves in other areas, including mitochondrial DNA (mtDNA) haplotypes that vary in frequency between wolves in Southeast Alaska and other North American locations (Leonard et al. 2005; Muñoz-Fuentes et al. 2009, 2010; Weckworth et al. 2010, 2011). Analysis of 12 nuclear microsatellite loci show allele frequency differences between wolves in Southeast Alaska and wolves in the continental areas, although there are few private alleles in Southeast Alaska (Weckworth et al. 2005). An extensive study of variation of 14 microsatellite loci of 1923 North American wolves also showed differentiation of Southeast Alaska from other populations (Carmichael et al. 2007, 2008), although cluster analyses grouped wolves from Southeast Alaska, interior Alaska, and northwestern Canada together. An analysis of 48 036 SNP showed genetic differentiation of coastal British Columbia wolves and North American continental populations (vonHoldt et al. 2011), although this analysis included only 3 coastal British Columbia wolves. Knowles (2010) described variation at 26 221 SNP that showed differentiation of 155 wolves from interior Alaska and Canada and 10 wolves from Southeast Alaska.

These studies indicate there is genetic differentiation of wolves in Southeast Alaska and wolves in other areas. However, the numbers of wolves sampled and loci assessed have been limited. New methods developed in dogs (i.e., SNP) allow us to expand upon these studies with more loci and larger sample sizes to better quantify the genetic variation of
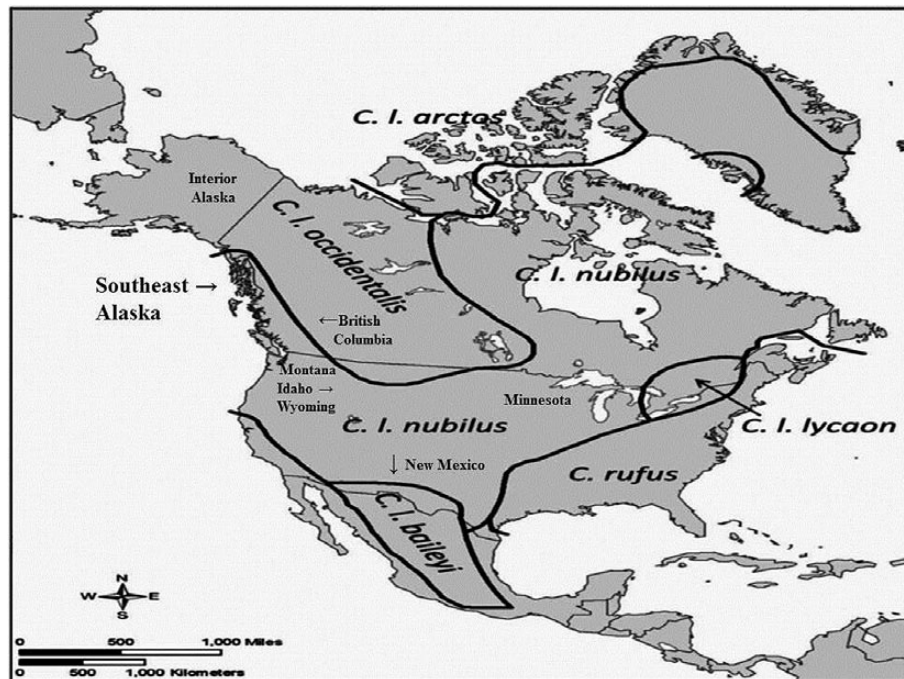

Figure 1. North American wolf (*Canis lupus*) sampling locations and subspecies distribution (Adapted from Nowak (1995, 2002) and Chambers et al. 2012).

wolves in Southeast Alaska as has been done elsewhere (von-Holdt et al. 2011). In this article, we describe the population genetics of wolves, coyotes, and dogs with SNP genotype data obtained using the Illumina170K CanineBeadChip. Our primary objective is to quantify the genetic differentiation of wolves within southeast Alaska and among wolves, coyotes, and dogs in several areas in North America, with a secondary objective to assess the data with regard to taxonomy and management.

Materials and Methods

Sample Collection

We collected tissue samples (skin, hair, muscle, blood) from 431 wolves, coyotes, and dogs from locations across North America (Table 1) including wolves from 6 Game Management Units (GMU) in Southeast Alaska: GMU1A (including the southernmost mainland and Revillagigedo Island), GMU1B (mainland north of GMU1A), GMU1C (mainland north of GMU1B), GMU1D (mainland north of GMU1C), GMU 2 (Prince of Wales Island), and GMU3 (including Kupranof, Etolin, Mitkof islands, Supplementary

Figure 1 online). Maps of Alaska GMU are available at http://www.adfg.alaska.gov/index.cfm?adfg=huntingmaps.bygmu. All of the wolf and coyote samples were obtained from legal harvest or animal control and research. We refer to the populations by geographic locations shown in Table 1. All of the samples are from wild coyotes and wolves, except the New Mexico wolves spent part of their lives captive and part of their lives wild, and are descendants of a captive-bred population started with 7 founders from Mexico (M. Dwire, US Fish and Wildlife Service, personal communication; Hedrick and Fredrickson 2008). We refer to wolves from the 6 Southeast Alaska locations collectively as Southeast Alaska wolves, and wolves from interior Alaska, Montana, Idaho, and Wyoming as northern wolves. Wolves from Idaho, Montana, and Wyoming are considered northern wolves because they originated from animals transplanted from the northern wolf (*C. l. occidentalis*) range in Alberta and British Columbia (Forbes and Boyd 1996, 1997). Wolves in interior Alaska are also considered northern wolves (Chambers et al. 2012). The wolves from British Columbia include 1 wolf from Vancouver Island and 34 from areas east of the Coast Mountain Range. Wolves from British Columbia are considered *C. l. occidentalis, C. l. nubilus, or C. l. ligoni* (Chambers et al.

Table 1 Numbers and locations of samples and observed heterozygosity of wolves, coyotes, and dogs that were genotyped with the Illumina170K CanineBeadChip

Species	Population name	Location	Subspecies/breed	Heterozygosity	Number	Total
Wolf	SEAK[a] wolf	GMU2 Southeast Alaska[b]	*Canis lupus nubilus* or *ligoni*	0.190	55	305
	SEAK wolf	GMU3 Southeast Alaska	*Canis lupus nubilus* or *ligoni*	0.181	38	
	SEAK wolf	GMU1A Southeast Alaska	*Canis lupus nubilus* or *ligoni*	0.195	22	
	SEAK wolf	GMU1B Southeast Alaska	*Canis lupus nubilus* or *ligoni*	0.193	8	
	SEAK wolf	GMU1C Southeast Alaska	*Canis lupus nubilus* or *ligoni*	0.230	13	
	SEAK wolf	GMU1D Southeast Alaska	*Canis lupus nubilus* or *ligoni*	0.259	2	
	BCWolf	British Columbia Canada	*Canis lupus nubilus* or *occidentalis*	0.244	35	
	INTAK[c] wolf	Interior Alaska	*Canis lupus occidentalis*	0.239	40	
	WY[c] wolf	Wyoming	Transplanted *C. l. occidentalis*	0.276	25	
	ID[c] wolf	Idaho	Transplanted *C. l. occidentalis*	0.270	23	
	MT[c] wolf	Montana	Transplanted *C. l. occidentalis*	0.251	16	
	MN wolf	Minnesota	*Canis lupus lycaon* or *nubilus*	0.237	20	
	NM wolf	New Mexico	*Canis lupus baileyi*	0.172	8	
Coyote	AK Coyote	Alaska	*Canis latrans*	0.131	2	35
	CT Coyote	Connecticut	*Canis latrans*	0.372	1	
	AZ coyote	Arizona	*Canis latrans*	0.152	15	
	ME coyote	Maine	*Canis latrans*	0.281	4	
	WY Coyote	Wyoming	*Canis latrans*	0.212	6	
	NE coyote	Nebraska	*Canis latrans*	0.165	1	
	NV coyote	Nevada	*Canis latrans*	0.157	1	
	WI coyote	Wisconsin	*Canis latrans*	0.177	2	
	CO coyote	Colorado	*Canis latrans*	0.162	1	
	MS coyote	Mississippi	*Canis latrans*	0.228	2	
Dog	MtTx	Montana and Texas	*C. familiaris* Australian shepard, Border collie	0.334	2	91
	Mixed		*Canis familiaris* Mixed breed	0.351	36	
	Poodle		*Canis familiaris* Poodle	0.324	53	
Total						431

[a]Southeast Alaska.

[b]GMU = Alaska Game Management Unit.

[c]Northern wolves.

2012), and are geographically adjacent to Southeast Alaska so we treated them as a separate group.

DNA Extraction and Genotyping

We isolated DNA from wolf and coyote skin, muscle, and blood using the Qiagen DNeasy 96 Blood & Tissue Kit and (Qiagen, MA, USA) and the MagMAX™ Sample Preparation System (Life Technologies Inc., Carlsbad, CA). DNA from the dog samples was extracted from blood collected in EDTA anticoagulant with the Gentra Puregene kit (Qiagen) as described by Rincon et al. (2011). A total of 431 samples from wolves ($N = 305$), coyotes ($N = 35$) and dogs ($N = 91$) were genotyped for 173 662 SNP loci using the Illumina170K CanineBeadChip (Geneseek Inc., Lincoln, NE).

Quality control and filtering of the 173 662 genotypes for each sample was performed with the SNP Variation Suite (SVS) software version 7 (Golden Helix Inc., Bozeman, MT). We removed SNP with a call rate <0.85, number of alleles > 2, and minor allele frequency (MAF) < 0.05 (i.e., we considered loci as polymorphic with a MAF > 0.05 criterion, e.g., Chakraborty et al. 1980; Clark et al. 1981). We analyzed linkage disequilibrium (LD) with the SVS LD pairwise analysis module with LD pruning and CHM computation with an r^2 threshold of 0.99, and removed 1 of each pair of linked loci from the analysis. We calculated observed heterozygosity (H_o) for each population (Table 1) for the autosomal SNP loci using SVS.

Population Genetic Analysis

We assessed differentiation of populations and species with 3 methods: individual genetic distances and Principal Components Analysis (PCoA, Orloci 1978); population genetic distances (F_{st}, Weir and Cockerham 1984) and Neighbor-Joining cluster analysis (NJ, Saitou and Nei 1987); and maximum likelihood estimation of individual animal ancestries (ADMIXTURE v. 1.23, Alexander et al. 2009).

We did PCoA of pairwise individual genetic distances with SVS. The PCoA analysis shows the individual animal relatedness graphically in multidimensions. The PCoA was performed under assumptions of an additive model and a dominant model. Output data for P–P/Q–Q plots showed the additive model was a better fit for the PCoA analyses (Supplementary Figure 2 online). Separate PCoA analyses were done for all 3 species (wolf, coyote, and dog) and for only wolves.

To quantify differentiation of allele frequencies among the wolf, coyote, and dog populations we calculated pairwise F_{st} between the populations in Table 1 with sample sizes > 1. The fixation index algorithm was performed by calculating genetic distance based on all markers (after quality control and filtering) using Cochran–Mantel–Haenszel statistics (CMH test) with SVS. NJ analysis with no rooting and midpoint rooting of the pairwise F_{st} values was done with MEGA version 5.2 (Tamura et al. 2011). NJ analysis was also done with the individual animal genetic distances with the neighbor program in PHYLIP (Felsenstein 2004) and an unrooted consensus tree was generated with the majority rule option

in the consense program in PHYLIP as was done with other SNP data in dogs, coyotes, and wolves (vonHoldt et al. 2010). We also compared the mean F_{st} within and between groups of populations including all wolves, Southeast Alaska wolves, and northern wolves with a 2-tailed z test of the means ($\alpha = 0.05$). This quantifies whether differences of allele frequencies (measured as F_{st}) among populations within a group (e.g., within Southeast Alaska) are greater or less than differences between that group and others (e.g., between Southeast Alaska and northern wolves), and therefore if populations within a group are genetically homogeneous relative to intergroup differentiation (Ramey et al. 2005; Cronin et al. 2013).

We also quantified the differentiation of the populations in Table 1 with maximum likelihood estimation of individual animal ancestries with ADMIXTURE v. 1.23 considering numbers of assumed clusters (K values) from 2 to 28. Consistent results were obtained by using the lowest standard error estimated with the cross validation method (Alexander et al. 2009).

Results

Of the 173 662 SNPs analyzed, 125 595 SNP passed the quality control analysis and resulted in reliable genotypes of wolves, dogs, and coyotes (SNP genotype data have been deposited in the Dryad data archive). The other 48,067 SNP were removed from the analysis. LD pairwise analysis of the 125 595 SNP resulted in 1794 pairs in LD. One of each linked pair was removed from the analysis resulting in a final set of 123 801 SNP that were used in the population genetic analysis. Heterozygosity was generally higher in dogs (> 0.3) than in wolves (0.17–0.28) and coyotes (0.13–0.37, Table 1).

Population Genetic Analyses

PCoA considering genetic distances between individuals (Supplementary Table 1 online) shows graphically the interspecies and interpopulation relationships for the 123 801 SNP loci (Supplementary Figure 3 online). Considering all 3 species, the first 2 coordinate axes explained 75% of the variation of the genetic distances and show dogs in a separate cluster from wolves and coyotes. Within the dog cluster, the mixed breed dogs cluster separately from the poodles. Some wolves occur close to or overlap with coyotes in the PCoA graph, particularly some wolves from Idaho, Montana, and Wyoming and coyotes from Maine. Considering only the wolf populations, the first 2 coordinate axes explained 62% of the variation of the genetic distances in the PCoA analysis. This analysis indicates that the individual genetic distances of Southeast Alaska wolves vary among the GMU with overlap of adjacent GMU: GMU1A, 1B, 1C, and 1D on the mainland; and GMU2 and GMU3 on islands to the west (Figure 2). The PCoA analysis shows the wolves in Southeast Alaska GMU1C and GMU1D overlap with British Columbia and the northern wolf populations. The New Mexico, Interior Alaska, Minnesota, Montana, Wyoming, and Idaho wolves overlap extensively. Some of the Montana, Wyoming, and Idaho wolves and 1 British
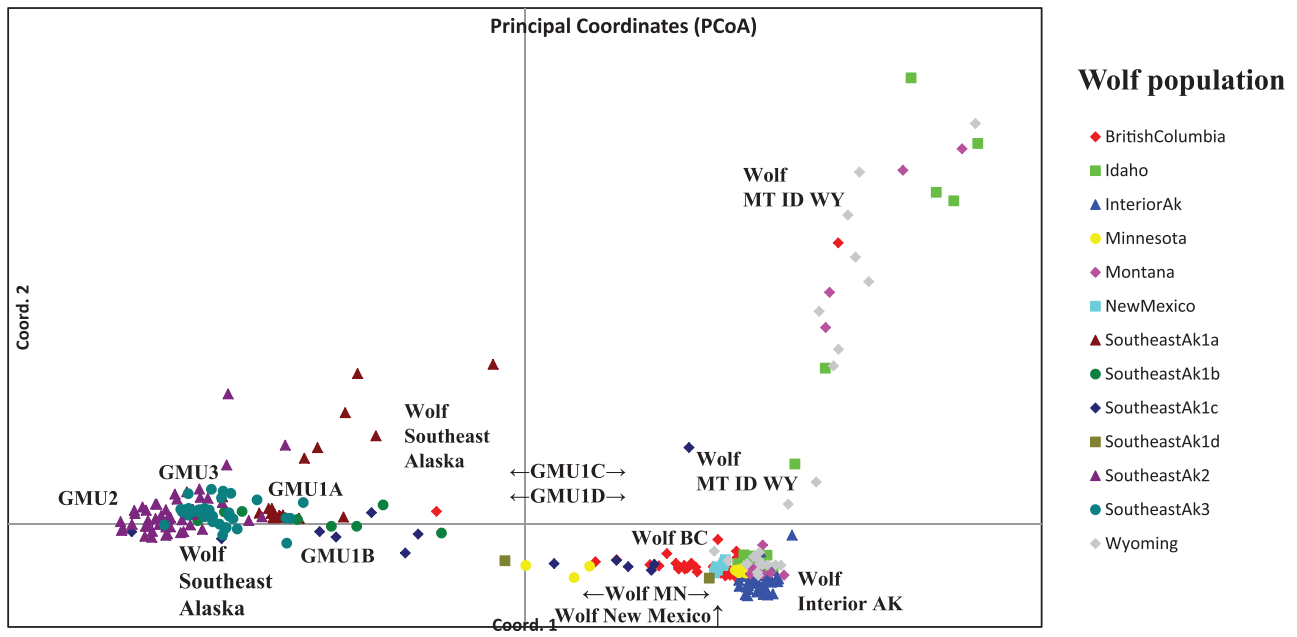
Figure 2. Graph of first 2 coordinate axes of principal components analysis (PCoA) of wolf individual genetic distances for 123 801 single nucleotide polymorphism (SNP) loci.

Columbia wolf occur scattered outside the primary clusters of wolves.

The ADMIXTURE analysis was performed considering $K = 2$ to 28. Cross validation values ranged from 0.418 ($K = 14$) to 0.529 ($K = 2$). The ADMIXTURE analysis indicates a best fit of $K = 14$ showing the lowest cross validation error value (0.418, Supplementary Figure 4 online). However, low cross validation values from 0.420 to 0.429 were also identified for $K = 9$, 10, 11, 12, 15, 16, and 17, so the number of clusters defining the populations is not definitive. The ADMIXTURE clusters with $K = 14$ contain groups with probabilities of inclusion > 75% (Supplementary Table 2 online) including: cluster 6 coyotes from all locations except Maine and Connecticut; cluster 4 mixed breed dogs; cluster 2 Minnesota wolves; cluster 12 New Mexico wolves; and cluster 5 Southeast Alaska GMU3 wolves (Figure 3). Poodle dogs occurred in 3 clusters (1, 4, and 7) with probabilities of inclusion between 10% and 61%. Interior Alaska wolves were predominant in cluster 13 (probability = 74%), and also occurred in cluster 10 (probability = 23%) with Wyoming, Montana, Idaho, and British Columbia wolves (probability = 13–65%). Wyoming, Montana, Idaho, and British Columbia wolves were also common in cluster 14 (probability = 19–64%). Maine and Connecticut coyotes were common in several clusters (probability = 13–47%) including cluster 6 with the other coyotes, clusters 8 and 14 with Wyoming, Montana, and Idaho wolves, and clusters 9 and 11 with Southeast Alaska wolves.

The Southeast Alaska wolves from different GMU were common in different ADMIXTURE clusters: wolves in GMU3 in cluster 5 (probability = 85%); wolves in GMU1C and GMU2 in cluster 9 (probability = 21–50%); wolves in GMU2 in cluster 11 (probability = 41%); wolves in GMU1D in cluster 13 (probability = 27%); and wolves in GMU1A and GMU1B, in cluster

3 (probability = 24–63%). Wolves in GMU1C and GMU1D were also common in cluster 10 (probability = 37–44%) as were British Columbia (65%) and Interior Alaska (23%) wolves. Wolves from all the Southeast Alaska GMU occurred in cluster 5 with a wide range of probabilities: 6% (GMU2), 14% (GMU1C), 20% (GMU1D), 23% (GMU1A), 55% (GMU1B), 20% (GMU1D), and 85% (GMU3).

The F_{st} values (Supplementary Table 3 online) show greater interspecies differentiation of allele frequencies of wolves, dogs, and coyotes (mean $F_{st} = 0.2790$–0.3379) than the intra-species differentiation among populations, although there is overlap of the ranges of the inter- and intra-species F_{st} values (Table 2). Wolves, dogs, and coyotes are in different clusters, and coyotes and dogs cluster separately from wolves in the NJ tree (Figure 4). This topology occurs in either an unrooted tree or a mid-point rooted tree, and regardless of which coyote and dog populations are included or excluded from the NJ analysis. This is unexpected because wolves and dogs are thought to be monophyletic relative to coyotes (Vilà et al. 1999; Wayne and vonHoldt 2012). However, the individual animal genetic distances (used in the PCoA analysis) were also subjected to NJ analysis and in this case wolves and dogs cluster together, separately from coyotes (Supplementary Figure 5 online).

The NJ analysis of F_{st} indicates the wolves in Southeast Alaska cluster together, and close to wolves from British Columbia and interior Alaska (Figure 4). The most northerly group of wolves in Southeast Alaska (GMU1D) clusters with wolves in interior Alaska, outside the cluster with the other Southeast Alaska and British Columbia populations, although the small sample size ($N = 2$) for GMU1D makes this result preliminary. The wolves in GMU2 and GMU3 co-occur on a terminal branch of the NJ tree. Wolves from the northern
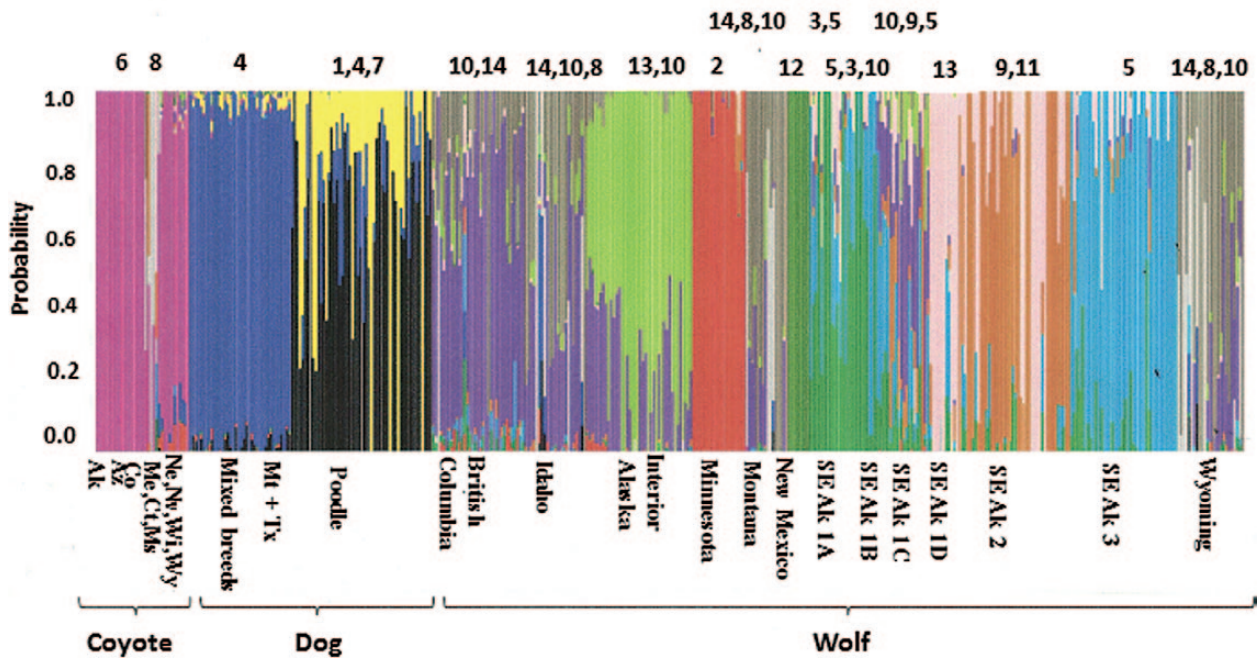
Figure 3. Histogram of ADMIXTURE analysis for $K = 14$ of 123 801 SNP genotypes for 431 wolves, coyotes, and dogs.

Rocky Mountain States (Montana, Idaho, Wyoming) cluster together, and wolves from Minnesota and New Mexico cluster on a separate branch of the NJ tree. The same tree topology of wolf populations is obtained in NJ analyses with either an unrooted tree or a mid-point rooted tree, and with or without dogs and/or coyotes included.

Interpretations of the PCoA and ADMIXTURE graphs and the NJ tree are somewhat subjective so we did a quantitative assessment of the differentiation of populations as indicated by F_{st}. Comparisons of F_{st} indicate the level of differentiation among the locations within Southeast Alaska is comparable to differentiation between Southeast Alaska and populations from other geographic areas (Table 2). Differentiation among the 6 Southeast Alaska locations (mean $F_{st} = 0.1268$ is not significantly different from the mean F_{st} among all of the wolf populations sampled across North America (mean $F_{st} = 0.1525$, $P = 0.22$), the mean F_{st} between Southeast Alaska and British Columbia (mean $F_{st} = 0.1195$, $P = 0.79$), or between Southeast Alaska and northern wolves ($F_{st} = 0.1501$, $P = 0.29$). The differentiation among locations within Southeast Alaska is significantly less than the differentiation of Southeast Alaska and Minnesota wolves ($F_{st} = 0.2012$, $P = 0.007$), and of Southeast Alaska and New Mexico wolves ($F_{st} = 0.3448$, $P < 0.0001$). Differentiation between wolves in GMU2 and the other 5 Southeast Alaska locations (mean $F_{st} = 0.1511$) is not significantly different than that among the other 5 Southeast Alaska locations (mean $F_{st} = 0.1147$, $P = 0.36$).

The other group for which intragroup and intergroup variation can be compared is northern wolves. Differentiation of SNP allele frequencies among locations of the northern wolves (mean $F_{st} = 0.0451$) is significantly less than that within Southeast Alaska ($F_{st} = 0.1268$, $P = 0.0001$), between all wolf populations ($F_{st} = 0.1525$, $P < 0.0001$), between wolves in Southeast Alaska and northern wolves ($F_{st} = 0.1501$, $P < 0.0001$), between northern wolves and Minnesota wolves ($F_{st} = 0.1154$, $P < 0.0001$), between New Mexico wolves and northern wolves ($F_{st} = 0.2330$, $P < 0.0001$); and not significantly different than the mean F_{st} between northern wolves and wolves from British Columbia ($F_{st} = 0.0390$, $P = 0.63$). This indicates that SNP differentiation among populations of northern wolves is low relative to differentiation among areas within Southeast Alaska, and northern wolves and British Columbia wolves are a relatively homogeneous group.

For the comparisons of populations with only 1 sampling location (i.e., no z-test because there is no mean or variance) there is relatively high F_{st} between Minnesota wolves and New Mexico wolves ($F_{st} = 0.2527$) and British Columbia and New Mexico wolves ($F_{st} = 0.2219$) compared with the F_{st} among all the wolves sampled across North America ($F_{st} = 0.1525$). These relationships are shown graphically in the NJ tree (Figure 4) in which wolves from Southeast Alaska occur in a cluster with wolves from British Columbia and northern wolves, while Minnesota wolves and New Mexico wolves cluster separately.

Discussion

In our study and others (Vaysse et al. 2011) the Illumina170K CanineBeadChip enabled extensive genotyping of wolves and coyotes, in addition to dogs for which it was developed. These SNP data represent variation across the canid genome

Table 2 Summary of F_{st} values derived from 123,801 SNP genotypes among wolf, coyote, and dog populations with $N > 1$

Populations compared	Fst Mean (SE)[a]	Fst Range	Fst 95% CL[b]
Intraspecies			
Among groups of dogs	0.0843 (0.0350)	0.0145–0.1251	0.1509
Among coyote locations	0.1105 (0.0211)	0.0188–0.2927	0.0453
Among all wolf locations	0.1525 (0.0101)	0.0124–0.3903	0.0202
Comparisons of southeast Alaska wolves			
Among all 6 Southeast AK locations	0.1268 (0.0184)	0.0344–0.2811	0.0394
Among 5 Southeast AK locations excluding GMU2	0.1147 (0.0225)	0.0344–0.2463	0.0508
GMU2 versus 5 other Southeast AK locations	0.1511 (0.0326)	0.1139–0.2811	0.0904
Southeast AK versus BC wolves	0.1195 (0.0215)	0.0607–0.1849	0.0552
Southeast AK versus MN wolves	0.2012 (0.0206)	0.1436–0.2638	0.0529
Southeast AK versus northern wolves (interior AK, ID, MT, WY)	0.1501 (0.0122)	0.0430–0.2441	0.0252
Southeast AK versus NM wolves	0.3448 (0.0158)	0.2810–0.3903	0.0407
Comparisons of North American populations			
Among northern wolves (interior AK, ID, MT, WY)	0.0451 (0.0109)	0.0124–0.0784	0.0279
MN wolves versus northern wolves (interior AK, ID, MT, WY)	0.1154 (0.0068)	0.0981–0.1271	0.0217
MN wolves versus NM wolves	0.2527 (N/A)	N/A	N/A
Northern wolves (interior AK, ID, MT, WY) versus NM wolves	0.2330 (0.0102)	0.2065–0.2526	0.0325
Comparisons with BC[c]			
BC wolves versus NM wolves	0.2219 (N/A)	N/A	N/A
BC wolves versus northern wolves (interior AK, ID, MT, WY)	0.0390 (0.0065)	0.0240–0.0535	0.0208
BC wolves versus Northern Rockies wolves (ID, MT, WY)	0.0342 (0.0062)	0.0240–0.0454	0.0267
BC wolves versus MN wolves	0.1031 (N/A)	N/A	N/A
Interspecies			
Dog versus coyote	0.3198 (0.0185)	0.2061–0.5222	0.0391
Dog versus wolf	0.3379 (0.0113)	0.2228–0.5153	0.0228
Wolf versus coyote	0.2790 (0.0111)	0.0817–0.4480	0.0221

[a]Mean (and standard error) values of pairwise F_{st} (Supplementary Table 3 online) of the groups identified in Table 1 with $N > 1$.
[b]95% Confidence level of the mean.
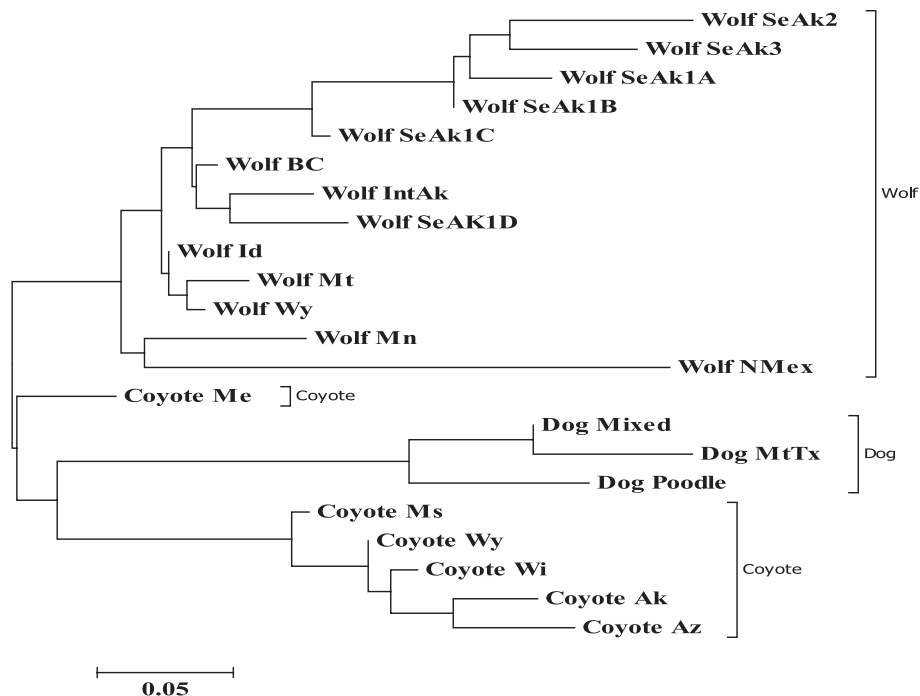[c]BC (British Columbia).

Figure 4. Neighbor-Joining tree of pairwise F_{st} values derived from 123 801 SNP genotypes among wolf, coyote, and dog populations identified in Table 1 with N > 1.

including coding and noncoding regions, synonymous and nonsynonymous substitutions, and not specifically gene regions that are under selection or selectively neutral. We found greater heterozygosity in dogs than in wolves or coyotes, which may be because of ascertainment bias. The SNP were discovered in comparisons of dog breeds and may be biased against wolf-specific SNP (Vaysse et al. 2011). Other assays of SNP developed in dogs had similar levels of variation in dog, wolf, and coyote but lower variation in more distantly related canids (vonHoldt et al. 2011). The levels of observed heterozygosity we report (Table 1) are comparable to those for wolves (0.12–0.25) and coyotes (0.14–0.20) and higher than those for dogs (0.24) reported by vonHoldt et al. (2011) for 48 036 SNP. The New Mexico wolves we sampled have low heterozygosity compared with other wolves, perhaps reflecting their origin from only 7 founding animals (Hedrick and Fredrickson 2008); and Maine and Connecticut coyotes have high heterozygosity compared with other coyotes, perhaps reflecting their mixed coyote–wolf ancestry (Kays et al. 2010). Among the Southeast Alaska wolves, those from GMU1D had the highest heterozygosity suggesting that SNP variation was adequately represented there despite a sample size of $N = 2$.

Interspecies Relationships

Phylogenetic analyses show that dogs and wolves share a more recent common ancestry than either does with coyotes (Bardeleben et al. 2005; vonHoldt et al. 2011; Wayne and von-Holdt 2012). However, there are contrasting relationships of these 3 species depending on the genetic markers and analysis used. For example, our SNP data resulted in a NJ tree (Figure 4) in which dogs cluster with coyotes separately from wolves. Phylogenetic analyses of 6 nuclear gene sequences also group dogs and coyotes separately from wolves (Figure 1 of Bardeleben et al. 2005) while PCoA of 94 SNP genotypes showed wolf and coyote overlapping in a cluster separate from dogs (Figure 3 of Gray et al. 2010). In contrast, NJ analysis of individual animal genetic distances derived from SNP (Supplementary Figure 5 online, vonHoldt et al. 2010), microsatellite genetic distances (García-Moreno et al. 1996), and phylogenetic analysis of mtDNA and nuclear gene sequences (Vilà et al. 1997, 1999; Bardeleben et al. 2005; Gray et al. 2010; Wayne and vonHoldt 2012) show that wolves and dogs cluster together, separately from coyotes. This indicates that caution is advisable when interpreting genetic relationships with molecular markers.

Wolves, coyotes, and dogs are known to hybridize in captivity and the wild (e.g., Vilá et al. 1997; Wayne and Vilá 2003; Hailer and Leonard 2008; Kays et al. 2010; Monzón et al. 2014). Assessment of interspecies hybridization is beyond the scope of our study, but we note that the coyotes in the northeast United States (i.e., Maine and Connecticut) have relatively low probability in the ADMIXTURE cluster 6 that has a high probability for all of the other coyote populations (Supplementary Table 2 online), and they cluster separately from other coyotes in the NJ tree (Figure 4) and PCoA graph (Supplementary Figure 3 online). This may be due to some

wolf ancestry in northeast US coyotes due to hybridization (Kays et al. 2010; Monzón et al. 2014).

Variation Within Southeast Alaska

Wolves in Southeast Alaska are not a genetically homogeneous group and there is as much or more genetic differentiation among locations within Southeast Alaska as there is between other areas (Table 2). This includes significantly more differentiation among areas in Southeast Alaska than among populations of northern wolves in interior Alaska and the northern Rocky Mountain states. This pattern is likely due to the recent post-glacial colonization of Southeast Alaska, and island and mountainous geography resulting in limited gene flow within Southeast Alaska and between Southeast Alaska and other regions (Weckworth et al. 2005, 2010, 2011; Carmichael et al. 2007, 2008).

The SNP variation among locations in Southeast Alaska shows different relationships, depending on the analysis used. For example, in the PCoA graph and NJ tree (Figures 2 and 4) GMU3 and GMU2 appear closely related, while wolves from these locations predominate in different clusters in the ADMIXTURE analysis (Figure 3). These differences reflect the different assumptions and characteristics of each method. However, the mean F_{st} between the wolves in GMU2 and the other 5 Southeast Alaska locations is not significantly different than the mean F_{st} among the other 5 locations. This indicates that although the wolves in GMU2 show a degree of differentiation (Weckworth et al. 2005) they are not particularly differentiated compared to the overall differentiation among the Southeast Alaska locations and our genetic data do not support the proposal that wolves in GMU2 are a distinct population segment (CBD 2011; USFWS 2014). In the PCoA and ADMIXTURE graphs the 4 GMU on the Southeast Alaska mainland (1A, 1B, 1C, 1D) overlap and GMU1A and GMU1B overlap with GMU2 and GMU3 indicating some level of gene flow and/or recent common ancestry among all of the areas in Southeast Alaska. These genetic data combined with data on demographics and movements can help managers understand the relationships of wolves in Southeast Alaska.

Variation of Wolves in Southeast Alaska and Other Geographic Areas

There is considerable differentiation of SNP allele frequencies between wolves in Southeast Alaska and wolves in other areas. However, the PCoA, NJ, and ADMIXTURE analyses indicate relatively low differentiation of wolves in northern Southeast Alaska (GMU1C and GMU1D) and British Columbia and interior Alaska, as observed for microsatellites (Carmichael et al. 2008). This suggests that Southeast Alaska may have been colonized by and/or have gene flow with wolves from areas to the north and east, as well as the south (Klein 1965; Cook et al. 2006; Weckworth et al. 2005, 2010). There is also relatively low differentiation of wolves in British Columbia and northern wolves, including wolves in the northern US Rocky Mountains that originated from transplants of northern wolves from British Columbia and

Alberta (Forbes and Boyd 1996, 1997). The SNP data indicate that wolves in Minnesota and New Mexico have relatively high allele frequency differentiation from other wolf populations, consistent with other SNP data (vonHoldt et al. 2011).

Taxonomy and Management

The subspecies taxonomy of wolves in Southeast Alaska is uncertain as some authors (Nowak 1995, 2002; Chambers et al. 2012) suggest wolves in Southeast Alaska and coastal British Columbia are *C. l. nubilus* (Figure 1), while others also use *C. l. ligoni* for wolves in this area (Weckworth et al. 2005, 2010, 2011; MacDonald and Cook 2009). *C. l. ligoni* has been proposed as an endangered subspecies in Southeast Alaska (CBD 2011; USFWS 2014), so this designation has important implications for both taxonomy and management. In this regard it is important to acknowledge that subspecies designations, including those of wolves, are generally subjective (Wayne and Vilá 2003; Zink 2004; Cronin 2006; Cronin and Mech 2009). It is also important to note that our analysis of allele frequency differentiation with clustering algorithms is not a phylogenetic analysis (Felsenstein 1982). Allele frequencies vary due to population genetic factors (mutation, drift, selection, gene flow), and not necessarily phylogeny. Because taxonomy is based on phylogeny (Mayr 1982; Avise and Ball 1990), such analyses may not be applicable to formal taxonomic designations. However, SNP data can provide relevant information regarding the extent of gene flow and ancestry of populations and hence aid in assessment of species and subspecies phylogeny (Decker et al. 2009, 2014; vonHoldt et al. 2011).

Our results and others show that wolves in Southeast Alaska differ in allele frequencies from wolves in other regions but do not comprise a homogeneous population and have recently colonized the region and/or have gene flow with other areas (Weckworth et al. 2005, 2010, 2011; Carmichael et al. 2007, 2008; Knowles 2010). MtDNA haplotype frequencies of wolves in Southeast Alaska also indicate a degree of isolation, but the haplotypes are not monophyletic and haplotypes of wolves in Southeast Alaska also occur in other areas (Leonard et al. 2005; Muñoz-Fuentes et al. 2009, 2010, Weckworth et al. 2010; Chambers et al. 2012). North American wolves in general have small sequence divergence of mtDNA haplotypes, a general lack of phylogeographic structure, and common episodes of isolation and admixture which is not surprising in a species that commonly disperses hundreds of kilometers (Vilà et al. 1999). These results indicate that wolves in Southeast Alaska are not a genetically isolated or monophyletic population, and do not support a subspecies designation of wolves in Southeast Alaska. Our data and those of vonHoldt et al. (2011) also show SNP differentiation of Mexican wolves (*C. l. baileyi*) from other North American wolves. However, extant and historic samples show that Mexican wolves lack mtDNA monophyly, share haplotypes with wolves in other areas and with coyotes, (Leonard et al. 2005; Hailer and Leonard 2008), and extant Mexican wolves came from only 7 founders that may have included

dog ancestry (although genetic data indicate this is improbable and/or of small genetic importance, García-Moreno et al. 1996; Hedrick et al. 1997). These factors indicate that designation of a Mexican wolf subspecies is of questionable validity. Indeed, North American wolf subspecies in general are questionable and have been described as arbitrary, typological, and an intergrading series of populations (Wayne and Vilá 2003 and references therein).

These observations for wolves indicate that it is worth heeding the admonition of Wilson and Brown (1953) that populations be designated by geographic area instead of subjective subspecies. This would make taxonomy more rigorous and has practical applications. Consider recent findings that several subspecies listed under the US Endangered Species Act are not supported by genetic data, including the coastal California gnatcatcher (*Polioptila californica californica*, Zink et al. 2013), the Preble's meadow jumping mouse (*Zapus hudsonius preblei*, Malaney and Cook 2013), the wood bison (*Bison bison athabascae*, Cronin et al. 2013), and the wolf subspecies discussed above. This indicates there is unwarranted taxonomic inflation of wildlife subspecies designations similar to unwarranted species designations (Zachos et al. 2013). For wildlife management the traditional use of geographic populations as management units, such as the GMU designations for wolf populations in Alaska, is more appropriate than subspecies. The scientific rigor of population genetics, systematics, and taxonomy, and their application to management and conservation, would be enhanced by implementation of this practice.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

Funding

Acknowledgments

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