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Rapid Evolution of Northeastern Coyotes

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Javier Monzón

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Javier Monzón

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree,

hereby recommend acceptance of this dissertation.

Daniel Dykhuizen – Dissertation Advisor
Distinguished Professor, Department of Ecology and Evolution

Catherine Graham – Chairperson of Defense
Associate Professor, Department of Ecology and Evolution

H. Resit Akçakaya
Professor, Department of Ecology and Evolution

Roland Kays
Research Associate Professor, Department of Forestry and Environmental Resources,
North Carolina State University

This dissertation is accepted by the Graduate School

Charles Taber
Interim Dean of the Graduate School

Abstract of the Dissertation

Rapid Evolution of Northeastern Coyotes

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The potential for rapid adaptive evolution is a subject of great interest in evolutionary biology. I took a population genomics approach to study the contemporary evolution of northeastern coyotes (*Canis latrans*) via hybridization and ecological specialization. I genotyped 96 single nucleotide polymorphisms (SNPs) in a broad geographic sample of 427 northeastern coyotes. First, I examined the prevalence, spatial distribution, and ecology of admixture. I found that northeastern coyotes form an extensive hybrid swarm with individuals being highly admixed with wolves (*C. lupus*) and dogs (*C. familiaris*). Coyotes in areas of high deer density are genetically more wolf-like, suggesting that natural selection for wolf-like traits may result in local adaptation at a fine geographic scale. Second, I investigated whether ecological factors can influence genetic structure in coyotes inhabiting the complex, fine-grained mosaic of different habitats characteristic of the Northeast. I found a cryptic genetic pattern consistent with the hypothesis of metapopulation structure conforming to a mosaic of forested, agricultural, and urban habitat types. High deer densities also explained a small but significant proportion of

genetic variation. Given the recency of the coyote range expansion into the Northeast, these findings demonstrate the rapid formation of ecological barriers to gene flow in a few generations. Lastly, I examined the molecular basis of local adaptation by analyzing five SNPs associated with ecologically important morphological traits. I provided the first documented evidence of any wild canids with homozygous mutant genotypes in these five SNPs. Coyotes with mutant genotypes are morphological outliers or peripheral individuals, indicating a clear association of morphological and genetic variation. A substantial reduction of gene flow across habitats is apparently mediated by the density of white-tailed deer, a main prey species; and strong diversifying selection is acting on the genetic architecture that underlies morphological traits related to predation. These results suggest that a localized area of high deer density is mediating morphological adaptation and ecological specialization in coyotes. This dissertation represents the most extensive genomic investigation of eastern coyotes, integrating landscape genetics, evolutionary ecology, and the emerging field of functional wildlife genomics.

To the three most important ladies in my life:

my lovely wife, Patricia;

my amazing mother, Alba Lucia;

and my precious daughter, Sofia Michelle.

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AMOVA	analysis of molecular variance
F_{ST}	the proportion of the genetic variance contained in a subpopulation relative to the total genetic variance
MCMC	Markov chain Monte Carlo
MCP	minimum convex polygon
MDS	multi-dimensional scaling
mtDNA	mitochondrial DNA
PCA	principal components analysis
PCoA	principal coordinates analysis
PCR	polymerase chain reaction
QDMA	Quality Deer Management Association
QTL	quantitative trait locus
SNP	single nucleotide polymorphism

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CHAPTER 1

GENERAL INTRODUCTION AND NATURAL HISTORY OF NORTHEASTERN COYOTES

The potential for rapid adaptive evolution is a subject of great interest in evolutionary biology. The last decade has seen a surge of studies documenting rapid evolutionary responses to habitat deterioration (Levinton *et al.* 2003; Williams & Oleksiak 2008), altered trophic cascades (Reznick *et al.* 2007), biological invasions (Phillips *et al.* 2006; Carroll 2008), harvesting (Allendorf & Hard 2009; Darimont *et al.* 2009), captivity (Frankham 2007), and climate change (Jump & Peñuelas 2005; Bradshaw & Holzapfel 2006). Hybridization can also produce swift evolutionary responses via the sudden influx of alleles from a divergent lineage (Seehausen 2004; Kays *et al.* 2010a). Moreover, anthropogenic disturbance is known to break reproductive barriers and induce hybridization between species (Lamont *et al.* 2003; Stronen *et al.* 2012).

Despite the importance of contemporary evolution for understanding and managing biodiversity, causal mechanisms remain obscure. For example, it is known that hybridization is common when a species undergoes a range expansion (Seehausen 2004), but little is known about the ecological conditions conducive to hybridization or the fate of hybrid individuals with varying degrees of admixture. The role of hybridization in generating adaptive genetic and phenotypic variation is gaining more recognition among zoologists (Seehausen 2004), but which alleles and which functional traits are targets for selection is largely unknown. Also, adaptive

radiations require ecological diversification, but the mechanisms behind the rapid formation of non-physical barriers to gene flow remain elusive.

The northeastern coyote (*Canis latrans* Say 1823) is an ideal organism through which one can address these important questions. The coyote is the new apex predator in the Northeast, having colonized the region in the last 90 years, following the extirpation of wolves in the late 1800s. Northeastern coyotes are morphologically, behaviorally, and genetically different from their western counterparts. Compared to western coyotes, northeastern coyotes have larger skulls and bodies (Way 2007), kill more large ungulates (Gompper 2002), and show no avoidance of forested habitats (Kays *et al.* 2008). Introgressive hybridization with eastern wolves (*C. lycaon* or *C. lupus lycaon*) may explain these observed differences (Mengel 1971; Kays *et al.* 2010a). In fact, the movement of coyotes into the Northeast did not occur until they hybridized. A more diverse genome, with genes from both species, likely allowed them to survive in new habitats, both forested and human-dominated. Collectively, the recent range expansion into a region rich with ecological opportunities for diversification, the history of hybridization with native wolves, and the evident phenotypic differentiation from western populations, makes the northeastern coyote a good model to study contemporary evolution. Such study is facilitated by the phylogenetic proximity of the coyote to the domestic dog (*Canis familiaris*), for which a plethora of genomic resources and a rich body of literature on the genetic basis of phenotypic variation exist.

In this dissertation, I took a population genomics approach to investigate the rapid evolution of northeastern coyote via hybridization, ecological differentiation, and morphological adaptation. I first give a brief overview of the natural history of *Canis latrans* in the Northeast. I refer to the Northeast as the northeastern quadrant of North America that was not in the historic

range of coyotes prior to the 20th century; the region includes Ohio and the US states east and north of it, and Ontario and the Canadian provinces east of it. I also provide a brief review of genomic investigations of wild canids.

Ecology of northeastern coyote

Range expansion and population ecology

The coyote is an almost ubiquitous member of North American Carnivora. It is presently found in all continental states, provinces, and territories of the United States and Canada, as well as in Mexico and Central America as far south as Panama. However, this current distribution is the result of a recent and dramatically rapid range expansion. Historically restricted to the open deserts and plains of central and western North America, the coyote has colonized almost the whole continent in the last 100 years, with few exceptions (e.g., New York City and Long Island). The eastward range expansion may have been facilitated by the total extirpation of eastern wolves and red wolves (*C. rufus*) and widespread deforestation associated with agricultural development in the early 20th century (Gompper 2002; Fener *et al.* 2005; Kays *et al.* 2010a). The range expansion into the Northeast advanced as two primary colonization fronts: the northern front through Ontario where coyotes hybridized with resident Great Lakes wolves, and the southern route through Ohio, where wolves were extirpated prior to coyote expansion (Parker 1995; Kays *et al.* 2010a, b).

Along with its geographic range expansion, the coyote also experienced a niche expansion. Given its origin in the open grasslands and aridlands of central and southwestern North America, it is surprising that the coyote, after a million years of inhabiting open landscapes, suddenly colonized whole new biomes, including tropical, temperate, and boreal

forests. However, the suitability of northeastern forests as habitat for coyotes has been called into question. Low densities, low body fat reserves, low parturition rates, and low fecundity in Canada's boreal forest (Poulle *et al.* 1995; Samson & Crête 1997; Dumond & Villard 2000) suggest that northeastern forests represent marginal habitat (Crête *et al.* 2001). Coyotes may be poorly adapted for hunting in dense forest despite high densities of prey (Richer *et al.* 2002). However, these populations were studied in eastern Quebec and New Brunswick shortly after they were colonized by coyotes. Colonizing populations tend to have low demographic rates and atypical demographic characteristics, as shown by a comparative study of colonizing coyote populations in eastern New Brunswick and Nova Scotia and a more established population in New Hampshire (Moore & Millar 1984). Another study of an older population that colonized New York's boreal Adirondacks showed that coyote abundance is positively related to the amount of forest cover (Kays *et al.* 2008). Together, these results hint at the possibility of rapid adaptation following their colonization of new biomes.

Landscape and spatial ecology

It has been reported that the home ranges of eastern coyotes are 100-200% larger than those of western coyotes (Patterson & Messier 2001), but this is not accurate. The average home range of non-juvenile, resident eastern coyotes is about 18 km^2, but there is much variation across habitat types and degrees of human development (Table 1.1). Coyotes in urbanized areas tend to have compact home ranges, whereas those in more pristine areas tend to have very large home ranges. The east vs. west comparison holds true only when studies in heavily forested eastern landscapes are considered to the exclusion of other landscape types. Still, the largest home range reported for an adult urban coyote in Cape Cod, Massachusetts, is within the range

of sizes typical for wilderness coyotes. It is not well understood why coyotes inhabiting developed landscapes tend to have smaller home ranges. Urbanized and agricultural landscapes may have a higher availability of resources per unit area (Way *et al.* 2002), but the complex combination of environmental pressures in human-dominated landscapes (e.g., hunting, dogs, roads, etc.) tends to perturb spatial and social dynamics (Atwood 2006). Even individuals with home ranges in developed areas undoubtedly have small forest patches to use for cover, rest, or movement. Interestingly, at a fine spatial scale, even "cropland" and "urban" coyotes tend to avoid areas of high human activity and instead use forested corridors extensively (Atwood *et al.* 2004; Way & Eatough 2006). At a broader scale, northeastern coyotes prefer disturbed forest with open canopies and abundant natural edges (Kays *et al.* 2008).

Evolution of northeastern coyote

Paleontology and historical biogeography

Canis latrans has been identified in the fossil record from the base of the Pleistocene (~2.6 MYA), but most of the specimens have been found in western North America. A few fossils from Florida, Indiana, Maryland, Mississippi, Pennsylvania, Virginia, and West Virginia have been identified as *latrans* (Nowak 2002). This indicates that the prehistoric distribution of *C. latrans* included eastern North America, at least the Southeast and the Mid-Atlantic. Nowak (2002) observed that small coyotes did not colonize the east until the early Rancholabrean, when the widespread New World lineage of *Canis* eventually evolved into the large *C. armbrusteri*, thereby opening an ecological niche for smaller *Canis*. By the late Rancholabrean, the *armbrusteri* lineage continued to increase in size until it culminated in the enormous dire wolf (*C. dirus*), thus opening more ecological space for the mid-sized red wolf (*C. rufus*) to colonize

the entire east coast, from Florida to Maine. After the small coyote and the large dire wolf disappeared from the east by the end of the Pleistocene, only the red wolf remained. Interestingly, when the latter was exterminated by humans in the 20th century, the coyote returned to the Southeast (Nowak 2002).

In part, much of the coyote's recent range expansion represents a recolonization of its prehistoric and historic range. As just described, coyotes occurred in the Southeast and Mid-Atlantic during the Pleistocene. Also, coyotes occurred as far south as Costa Rica during the Pleistocene and throughout the Holocene up to the 19th century (Hidalgo-Mihart *et al.* 2004). However, the expansion into northeastern North America beyond Pennsylvania and the Great Lakes is a novel colonization of the region and its varied habitats.

Morphology

Northeastern coyotes are significantly larger than their western and Midwestern counterparts (Gompper 2002; Way 2007; Kays *et al.* 2010a). Male northeastern coyotes (mean = 16.4 kg) are larger than female northeastern coyotes (mean = 14.7 kg), but the latter are larger than both male (mean = 12.1. kg) and female (mean = 10.1. kg) coyotes from the West and Midwest (Way 2007). Given the swiftness of the range expansion into the Northeast, these size differences may represent a very rapid evolutionary process. Interestingly, coyotes in southern Ontario increased significantly in mean body weight and length from the 1960s to the mid-1980s (Schmitz & Lavigne 1987). This marked increase in body mass has important trophic consequences. According to Vezina's (1985) equation that relates predator and prey mass in mammalian carnivores, an increase of 39% in coyote body mass enables them to kill prey 46% larger. Although Bergmann's rule may explain the larger sizes of coyotes in the Northeast

relative to the Southwest, latitude only explained 13% of the variation in body size, whereas longitude explained more than 60% of the variation (Way 2007).

What else, beside geography, may explain the fact that northeastern coyotes are almost 40% larger? At least three plausible explanations may account for the observed differences in body size. Thurber and Peterson (1991) speculated that the larger size of northeastern coyotes was most likely a phenotypic response to enhanced food supply. Alternatively, Larivière and Crête (1993) argued that the larger size most likely represents an adaptive evolutionary response to the use of larger prey, namely white-tailed deer (*Odocoileus virginianus*). A third explanation for the larger northeastern coyotes is hybridization with wolves, although it is not mutually exclusive to the hypothesis of genetic adaptation to prey size. Lawrence and Bossert (1969) were the first to statistically analyze the skulls of northeastern coyotes and suggest that they have a *latrans* × *lupus* mixed ancestry. Silver and Silver (1969) raised northeastern coyotes in captivity together with western coyotes and noted that the former grew much larger. These results refute the phenotypic plasticity hypothesis and lend some support to the other two hypotheses, which imply a genetic basis for size differences. However, the results from captivity do not necessarily point to a selective process as the agent driving body size differentiation. In fact, as Mengel (1971) succinctly summarized, the Silvers (1969) and Lawrence and Bossert (1969) concluded that the large size of "New England *Canis*" resulted "not from strong selection favoring the rapid evolution of large predators, but rather from hybridization."

The second and third hypotheses may be combined to suggest that hybridization with wolves has introduced adaptive genetic variation. In other words, northeastern coyotes are intermediate in size between western coyotes and wolves because they have hybrid ancestry, but this mixed ancestry was advantageous for hunting large prey. The morphological and molecular

analyses of Kays *et al.* (2010a) support this blended adaptive hybridization hypothesis. It is important to emphasize that northeastern coyotes are not simply larger versions of their western counterparts, but that they are also more wolf-like. The skulls of northeastern coyotes show craniodental characteristics similar to wolves: they are proportionally broader and have large areas of attachment for masticatory muscles, suggesting a morphological specialization for killing large-bodied prey (Kays *et al.* 2010a).

Population genetics

Early studies in the 1950s and 1960s were highly suggestive of a hybrid origin for northeastern coyotes, long before the availability of any molecular data needed to confirm this. The hybridization hypothesis was proposed by various authors entirely on the basis of morphology (e.g., Lawrence & Bossert 1969) and captive rearing experiments. The successful breeding of F_1 coyote-dog hybrids (Silver & Silver 1969; Mengel 1971) and coyote-wolf hybrids (Kolenosky 1971) in captivity provided the first direct evidence of interspecific crosses resulting in viable and fertile offspring. The first molecular evidence to unequivocally confirm coyote-wolf admixture in the Great Lakes region and further east came in the early 1990s (Lehman *et al.* 1991; Wayne & Lehman 1992; Wayne *et al.* 1992; Roy *et al.* 1994). However, introgression initially appeared unidirectional, but in the direction contrary to theoretical expectations (Currat *et al.* 2008) that resident wolf genes would introgress the colonizing coyotes. These early genetic studies detected coyote-derived DNA in wolf populations, but no wolf-derived DNA in coyote populations, so biologists speculated that coyote-wolf mongrel offspring would only backcross with wolves. Only recently has evidence of wolf mitochondrial DNA introgressing northeastern coyotes been presented, first by Koblmüller *et al.* (2009) and Kays *et al.* (2010a).

Other recent studies demonstrated that northeastern coyotes have remained genetically distinct despite extensive admixture with eastern and western wolves (Rutledge *et al.* 2010; Wheeldon *et al.* 2010). Many subsequent molecular analyses debated over the taxonomic identity of the wolves in the Great Lakes region that hybridized with the advancing coyotes, but that remains outside the scope of this general introduction.

Initially, analyses of both mitochondrial and nuclear DNA variation did not detect substantial genetic structure among coyote populations across North America (Lehman & Wayne 1991; Roy *et al.* 1994). These continental-scale results were interpreted to suggest that high degrees of gene flow precluded differentiation even among very distant populations. Although coyotes are highly vagile mammals capable of long dispersal distances, the lack of evidence for population structure across their entire range was surprising. The appearance of homogeneity of *Canis latrans* across its range seems to be an artifact of small sample sizes, coarse geographic sampling, and few genetic markers. A much more geographically focused study of California coyotes demonstrated that cryptic genetic structure corresponded to habitat-specific breaks separating contiguous bioregions (Sacks *et al.* 2004). This study demonstrated that a better resolution may be attained with denser sampling and more molecular markers so that subtle genetic patterns can be detected. Despite the potential of fine-scale genetic structure in coyotes, little differentiation has been found among populations across the Northeast. For example, coyote populations from Maine, New York, New Brunswick, and southeastern Ontario were only slightly differentiated (Wilson *et al.* 2004; Way *et al.* 2010). Pairwise F_{ST} measures ranged from 0.011 to 0.045 in these studies, but they were not statistically evaluated against the null hypothesis, so it is difficult to interpret the biological significance of these measures which were attained with a small number of molecular markers. In the most extensive genetic investigation

of northeastern and Ohio coyotes in terms of sample size and geographic representation, Kays *et al.* (2010a) found three genetically distinct geographic subdivisions and irrefutable evidence of wolf introgression into the coyote gene pool. Still, their study only surveyed one molecular marker, the mitochondrial control region, which is maternally inherited and thus only provides a one-sided view into the complex ancestry of northeastern coyotes.

Studies examining genetic variation across a large number of loci in the genome in a large sample of individuals are sorely lacking. Additionally, sampling of a continuously distributed species like the coyote should not leave large gaps that may lead to the misinterpretation of genetic discontinuities (Schwartz & McKelvey 2009). The advent of genomic tools and new molecular markers, along with more rigorous statistical inference, can provide a fresh perspective into the relative contributions of different species to the ancestry of northeastern coyotes and the fine-scale separation of populations (Chambers 2010).

Genomics of wild canids

The development of genomic tools in model organisms has facilitated comprehensive surveys of neutral and adaptive variation in closely related non-model species. Kohn *et al.* (2006) coined the term "genome-enabled taxa" to describe species that benefit from the cross-taxon applicability of resources generated by genome projects. Since the genome of man's best friend, *Canis familiaris*, was sequenced (Lindblad-Toh *et al.* 2005), the coyote and other Canidae are now genome-enabled taxa. Here I give a brief, chronological review of studies that pioneered the use of dog genomic resources in molecular surveys of wild canids, highlighting their merits and weaknesses.

In one of the first demonstrations of SNP (single nucleotide polymorphism) discovery and genotyping in a population of wild canids, Seddon *et al.* (2005) used dog sequences to design assays for 24 SNPs to be interrogated in a wild wolf population. In this pioneering study, Seddon *et al.* identified individuals and their relationships, measured genetic diversity, compared the severely bottlenecked Scandinavian wolf population to the neighboring Finnish population, and identified immigrants. This study described a very laborious method of selecting and genotyping only a few SNPs (44 SNPs were identified from 25 sequence fragments, but only 24 SNPs were successfully genotyped), some of which had extremely low variability in the test population.

Andersen *et al.* (2006) later characterized a larger number of SNPs in an endangered population of Italian wolves. Andersen *et al.* discovered 59 SNPs by sequencing sites that were known to contain SNPs in domestic dogs; they genotyped 15 SNPs using the then-novel Pyrosequencing technology. They found some diagnostic SNPs that were polymorphic between wolves and dogs and may thus be useful in detecting dog-wolf hybrids. This study had a very small sample size (N = 14 Italian wolves), and described a very laborious method of selecting and genotyping non-independent SNPs: 59 SNPs were identified from 21 sequence fragments, out of 49 fragments that reliably amplified, from a test of 76 PCR primer pairs.

While previous investigations focused on using dog genomic resources to find SNPs in the closely related wolf, Sacks and Louie (2008) were the first to examine the utility of the dog genome for finding SNPs in distantly related non-model canids. Sacks and Louie used dog-derived primers to successfully amplify 48 SNP-rich regions in coyote, red fox (*Vulpes vulpes)*, and gray fox (*Urocyon cinereoargenteus*), the most ancestral of extant canids. This study demonstrated the utility of dog genomic tools to study genomic variation in any species of

Canidae. However, beside the very small sample size (nine red foxes, one gray fox, and one coyote), this study had low sequencing success (80%) because the authors did not test primers *in silico*, and low genotyping success (83%). Additionally, the fact that several SNPs were discovered per amplicon suggests they are tightly linked and not independent markers.

As SNPs quickly began to gain popularity among evolutionary biologists, Vali *et al.* (2008) introduced the use of short insertion-deletion polymorphisms (indels) for genetic studies of natural populations. Vali *et al*. devised a relatively simple algorithm for selecting indel markers from dog genome sequence data in GenBank and also a simple genotyping assay. They genotyped 94 indels in 5 natural wolf populations and 76 indels in the Scandinavian wolf population. This ground-breaking study demonstrated the utility of dog indels even in a population with low genetic diversity. Unfortunately, the method was only tested on a small sample of 18 wolves representing the global population and 27 wolves representing the Scandinavian population.

Molecular studies of Scandinavian wolves continued to advance the field of wildlife genomics. Hagenblad *et al.* (2009) raised the bar by genotyping 258 autosomal and X-linked microsatellites in 112 Scandinavian and 24 Russian wolves. With such a large number of markers and samples, Hagenblad *et al.* were able to conduct tests of selection. They found very high levels of linkage disequilibrium, a decrease in the rate of loss of diversity after an immigrant introduced new genetic material, and evidence for balancing and purifying selection at various loci. The study could have benefited from detailed pedigree data or at least a distinction between breeding and non-breeding individuals, which should be important because of the social structure of wolf packs.

Gray *et al.* (2009) continued to raise the bar by genotyping 106 SNPs in a large sample of 1001 canids (546 dogs, 344 wolves, 18 coyotes, and 93 distantly related jackals and foxes). Their multiplex genotyping method resulted in high amplification success, allowing Gray *et al.* to measure linkage disequilibrium, genetic structure, and ascertainment bias. Since a causal relationship exists between linkage disequilibrium and population history, Gray *et al.* were able to use the former to model the latter. By modeling demographic history using genomic data, they found evidence of a historical population contraction in two of five wild canid populations. All 106 SNPs were ascertained from 5 dog chromosomes, so the panel was not really representative of the whole genome, and only a fraction of loci were polymorphic in their coyote population.

vonHoldt *et al.* (2011) conducted the most extensive genomic survey of wild canids – and of any wild vertebrate taxon – to date. vonHoldt *et al.* used a SNP genotyping microarray (or SNP-chip) developed for the domestic dog to assay variation in over 48,000 loci in 912 dogs and 276 wild canids from six species worldwide. With this expansive dataset, they were able to address long-standing questions about diversification and admixture in red wolves, eastern wolves, and northeastern coyotes. Although the breadth of this genomic survey was, and still remains, unparalleled, the geographic sampling of coyotes was limited, with only 13 northeastern individuals, thus limiting inferences about admixture and population structure in northeastern coyotes.

Research objectives

In this dissertation, I took a population genomics approach to investigate the rapid evolution of northeastern coyote via hybridization, ecological differentiation, and morphological adaptation. Specifically, my objectives were to:

1. evaluate the prevalence, spatial distribution, and ecology of admixture in northeastern coyotes;
2. test whether geography, habitat variability, and admixture affect population genetic structure of northeastern coyotes;
3. examine the molecular basis of local adaptation by integrating molecular, morphological, and ecological data.

Selecting genetic markers

I genotyped a total of 108 SNPs, all ascertained from the dog genome project (Appendix A). In an exploratory analysis of the utility of dog SNPs for research in wild *Canis*, I used 16 SNPs chosen for their high heterozygosity in a subset of 15 northeastern coyotes. Heterozygosity is a measure of genetic variability, as is allelic richness with microsatellite markers. But, since all SNPs in this study were biallelic, it was important to select loci with a high observed heterozygosity to maximize the ability of each locus to resolve population structure. In order to evaluate the relative contributions of three putative parental populations of admixed northeastern coyotes, I used 63 ancestry-informative diagnostic SNPs. In order to assess population genetic structure in the region, I used the same 63 markers plus 28 more SNPs with high heterozygosity. Finally, in order to examine the molecular basis of rapid

morphological change, I used five SNPs that are quantitative trait loci associated with body and skull size in *Canis*.

Selecting individual samples

I generated genetic profiles for 509 northeastern canids, including 31 from Ohio, all archived in the mammal collection of the New York State Museum (Appendix B). All specimens were recent, the oldest being from 1999. I selected most samples (92%) from among those whose mitochondrial control region was sequenced by Kays *et al.* (2010a). The remainder consists of 35 samples included to maximize geographic representation within the region so as not to leave substantial sampling gaps. Fourteen of the samples were genotyped by vonHoldt *et al.* (2011) and served as positive controls.

Dissertation overview

The following chapters of this dissertation include four related studies (Chapters 2-5) and a general conclusion (Chapter 6). Chapter 2 was an exploratory project, in which I made the first attempt to evaluate genetic variation and population structure in northeastern coyotes using nuclear SNPs. While I was able to detect finer levels of population structure than previously reported, the SNP genotyping method I used for this pilot study was less than satisfactory. In Chapters 3-5, I used a different technology platform to genotype three different sets of SNPs in order to address questions of coyote-wolf admixture, ecological correlates of population structure, and local adaptation in morphology. Chapter 3 focuses on disentangling the complex admixed ancestry of northeastern coyotes. I used ancestry-informative diagnostic SNPs to show that all northeastern coyotes have some degree of recent ancestry from western wolves, eastern

wolves, and domestic dogs, and that variation in ancestry is related to prey densities. Chapter 4 focuses on delineating the spatial distribution of populations and finding ecological correlates of genetic differentiation. I used Bayesian and multivariate multiple regression analyses to show that population structure is affected by human land use and deer densities, and that parapatric populations are significantly differentiated despite the absence of physical movement barriers. Chapter 5 focuses on testing the long-standing idea of rapid morphological adaptation of northeastern coyotes. In this chapter, I integrated molecular data on genes of known function, morphological data, and ecological data to show that northeastern coyotes occur as locally adapted ecotypes that appear to be responding to varying concentrations of deer.

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Table 1.1. Annual home range size estimates of resident eastern coyotes inhabiting primarily urban, agricultural, and forested landscapes. Mean for each landscape type and grand mean are weighted by sample size, N.

	Home Range Size (km^2)				
Study Area	**Mean**	**Maximum**	**N**	**Method**	**Source**
URBAN					
Cape Cod, Massachusetts	33	52	6	95% MCP[a] vertex edited (breeders and associates)	Way *et al*. 2002
Albany, New York	8	15	14	95% fixed kernel (adults and yearlings)	Bogan 2004
Chicago, Illinois	5		84	95% MCP (adults and subadult residents)	Gehrt *et al.* 2009
Mean	**7**				
AGRICULTURAL					
Champlain Valley, Vermont	18	39	11	94% MCP (breeders and associates)	Person and Hirth 1991
Warren County, New Jersey	10	14	4	95% MCP	Eastman 2000
West-central Indiana	11	23	15	95% adaptive kernel	Atwood 2006
Mean	**13**				
FORESTED					
Eastern Maine	50	54	4	100% MCP (adults)	Caturano 1983
Eastern Maine	46	60	7	MCP removing outliers >3 km from previous or subsequent locations	Harrison *et al.* 1989
Western Maine	43		5	100% MCP	Major and Sherburne 1987
Acadia National Park, Maine	42	78	12	95% MCP	Winter 1990
Nova Scotia	63	105	13	95% adaptive kernel (composite home range of group members)	Patterson and Messier 2001
Mean	**50**				
Grand mean	**18**				

[a] MCP = minimum convex polygon

CHAPTER 2

BEYOND MITOCHONDRIAL DNA: A FIRST ATTEMPT TO EVALUATE POPULATION GENETIC STRUCTURE IN NORTHEASTERN COYOTES USING SINGLE NUCLEOTIDE POLYMORPHISMS

"Widely-ranging species, abounding in individuals, which have already triumphed over many competitors in their own widely-extended homes, will have the best chance of seizing on new places, when they spread into new countries. In their new homes they will be exposed to new conditions, and will frequently undergo further modification and improvement; and thus they will become still further victorious, and will produce groups of modified descendants."

- Charles Darwin, *On the Origin of Species* 1859

Abstract

Population structure plays an important role in evolution; yet detecting it largely depends on the type of genetic markers used, their variability in the population, and the spatial sampling scheme. I evaluated genetic variability and population structure in 385 eastern coyotes (*Canis latrans*) using 16 hypervariable single nucleotide polymorphisms (SNPs). Coyotes in Ohio are the most genetically diverse but northeastern coyotes also have a high level of heterozygosity. A region-wide analysis of population structure revealed three genetic populations, but these do not correspond to the same three subdivisions inferred in a recent analysis of mitochondrial DNA (mtDNA) sequences. More focused geographic analyses of population structure showed that Ohio and northeastern coyotes form their own panmictic populations, whereas coyotes in the intermediate contact zone, where two range expansion fronts meet, are highly structured. My

results suggest that studies based solely on mtDNA should be interpreted cautiously and demonstrate that genotyping several hypervariable SNPs in a dense geographic sample is an effective way to detect fine levels of population structure.

Introduction

Genetic structure is a ubiquitous property of natural, domesticated, and human populations. Population structure plays a considerable role in evolution, as both the basis and the consequence of local adaptation, adaptability of a species as a whole, and the splitting of one species into two (Wright 1949). The detection of genetic structure largely depends on the molecular markers assayed, their variability in the target population, and the spatial sampling scheme. Single nucleotide polymorphisms (SNPs) have become an increasingly popular and decreasingly expensive tool in the field of molecular population genetics. SNPs have properties that make them a superior alternative to other widely used genetic markers, such as microsatellites and mitochondrial DNA (mtDNA) sequences, in estimating genetic variation and detecting population structure (Morin *et al.* 2004).

Recent analyses of population structure in northeastern coyotes (*Canis latrans*) have revealed a general lack of genetic differentiation among sampling localities, except at the coarsest scales. Way *et al.* (2010) examined genetic variation and structure in a sample of coyotes from eastern Massachusetts using mtDNA and eight microsatellite loci. They found no genetic structure within Massachusetts or even within the region. Instead, northeastern coyotes seemed to make one uniform population slightly differentiated from western coyotes. In another recent analysis of genetic variation in eastern coyotes, Kays *et al*. (2010) inferred three coarse phylogeographic subdivisions: Ohio, the northeast zone, and a contact zone in western

Pennsylvania and New York where the colonization front from Ohio has spread into the northeastern population. Although Kays *et al.* surveyed genetic variation in a dense geographic sample of 686 coyotes, they only used one genetic marker, the mitochondrial control region. A genome-wide analysis of North American *Canis* detected population structure in *C. latrans*, but only at a broadest continental scale (vonHoldt *et al.* 2011). However, although vonHoldt *et al.* genotyped tens of thousands of SNPs, they only sampled 13 northeastern coyotes, making detection of finer levels of population structure in the region very improbable. Thus, a regional analysis of genetic variation in northeastern coyotes using many samples and many molecular markers is lacking.

In this study, I assessed genetic variation and population structure in northeastern coyotes using a dense geographic sampling scheme and several hypervariable SNPs. I hypothesized that population structure should be detectable at finer levels than in previous analyses by using a battery of high-heterozygosity SNPs and a spatially dense sample. My objectives in this study were to quantify genetic variability and population structure and to evaluate the efficacy of a medium-throughput method of genotyping SNPs ascertained from the dog genome.

Methods

Study area and sampling

The study area is located in northeastern North America and includes Ohio, Pennsylvania, New York, New Jersey, Connecticut, Massachusetts, Rhode Island, Vermont, New Hampshire, and southern Quebec (Figure 2.1). All samples (N = 385) are archived and vouchered in the New York State Museum, Albany, NY, and were collected with assistance of local hunters and trappers. Six samples came from previous scat surveys in New York

(Gompper *et al.* 2006; Kays *et al.* 2008). Because samples came from scat or animals killed for reasons other than research, I did not require IACUC review.

Marker selection and laboratory methods

I selected molecular markers based on a previous study that used the Affymetrix Canine Mapping Array to genotype 61,435 SNP loci in many wild and domestic canids, including 14 northeastern and 3 Ohio coyotes (vonHoldt *et al.* 2010; vonHoldt *et al.* 2011). I used the program PLINK (Purcell *et al.* 2007; http://pngu.mgh.harvard.edu/purcell/plink) to compute observed and expected heterozygosity per locus in the subset of 17 northeastern and Ohio coyotes. I selected 16 unlinked loci, each on a different chromosome, with the highest observed heterozygosity, a measure of genetic variability in a population (Table 2.1). These hypervariable SNPs allowed me to assess genetic variation and population structure in the larger target sample. I designed primers using the Primer3 software (http://frodo.wi.mit.edu/primer3) and tested them *in silico* against the dog CanFam2 genome assembly using the University of California, Santa Cruz In-Silico PCR web tool (http://genome.ucsc.edu).

I extracted total genomic DNA using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California) according to the manufacturer's instructions, and also used DNA from Kays *et al*. (2010). I determined final DNA concentrations using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, Delaware) and prepared four 96-well plates with genomic DNA aiming to attain a concentration of 5-30 ng/μL.

I genotyped 16 nuclear SNP markers in 378 coyote samples. SNP genotyping was done at the GenoSeq Core laboratory in the University of California, Los Angeles, using a high-resolution melting curve quantitative PCR method. The real-time PCR and melt curve analysis

were done on a LightCycler 480 thermal cycler (Roche, Inc.). DNA was amplified in a total volume of 10.5 µl, including 1 µl (5-30 ng) of genomic DNA, 0.5 µl of 4 µM primer mix, 1.75 µl of 25 mM $MgCl_2$, 2.25 µl of H_2O, and 5 µl of HiRes Melt master mix (Roche, Inc.). This PCR mix contains a dye that fluoresces when DNA is double-stranded. During the melt curve analysis the temperature increases very slowly to denature double-stranded DNA. Samples with variations in DNA sequence, even in one base pair, are distinguished by discrepancies in melt curve shape, thus discriminating each of the two homozygous and the heterozygous genotypes. I processed the raw data using the Gene Scanning module of the Roche LightCycler software, which automatically generates genotypes from the melt curves data. However, for quality control, I visually inspected all genotype calls and excluded samples that performed poorly or genotypes that were ambiguous.

Analyses of genetic variability and population structure

To the 378 samples genotyped in this study, I added 7 northeastern coyotes genotyped by vonHoldt *et al.* (2011). I used PLINK to calculate average observed and expected heterozygosity, measures of genetic variability, for the 16 SNPs in the three zones inferred by Kays *et al.* (2010) (Figure 2.1). In order to assess the ascertainment bias of using dog genome SNPs to study genetic variability and population structure in coyotes, I calculated average observed and expected heterozygosity in 5 subsets of canids from vonHoldt *et al.* (2010, 2011): northeastern coyotes, western coyotes, western wolves (*Canis lupus*), eastern wolves (*Canis lupus lycaon*), and dogs (*Canis familiaris*). I calculated heterozygosity using all 61,435 SNPs from the microarray because the vast majority of the loci in the microarray were ascertained by

dog-dog comparisons (vonHoldt *et al.* 2010), and again using only the 16 hypervariable SNPs genotyped in this study.

I used the program STRUCTURE (Pritchard *et al.* 2000) to infer the best number of genetic populations with nuclear SNPs. STRUCTURE implements a Bayesian algorithm to assign multilocus genotypes to genetic clusters by calculating the likelihood that a group of individuals constitutes a population. STRUCTURE makes clustering assignments without a priori inputs from the investigator that may bias the delineation of breeding populations. Despite STRUCTURE's ability to handle missing data, I excluded all individuals with more than five missing genotypes, resulting in 247 coyotes (16 in Ohio, 118 in contact zone, 113 in northeast zone). I tested whether finer population structure is detectable with the highly variable SNPs relative to the coarse structure detected with mtDNA. I analyzed all 247 coyotes together to test whether more than three populations are detectable at the regional level, and then analyzed each zone separately to test whether more than one population is detectable within each zone. For all analyses, I used 10,000 burn-in and 100,000 Markov chain Monte Carlo iterations, used the admixture ancestry model with correlated allele frequencies, and set the number of populations from K = 1 to K = 8.

Results

PCR amplification and genotyping success

I attempted to genotype 16 SNP loci in 378 coyotes for a total of 6,048 potential genotypes. However, 1,972 curves were non-interpretable and declared as "unknown." Despite attempting to optimize the PCR conditions by testing each primer pair in a small number of samples, the genotyping success rate was 67.3%. Post hoc analysis of the input genomic DNA

concentrations revealed that the high variability in the amplification and melt profiles may have been due to a high variance in DNA concentration across samples, even though I took care to follow Roche's recommendation to include 5-30 ng of genomic DNA into each PCR reaction.

Genetic variability and population structure

The overall sample of 385 eastern coyotes had lower genetic diversity than expected (Table 2.2), even though observed heterozygosity generally exceeded expected heterozygosity in the ascertainment panel of 17 northeastern and Ohio coyotes (Table 2.1). Ohio coyotes are the most genetically diverse in the region, but the eastward decay in genetic diversity observed with mtDNA is not replicated with nuclear SNPs. Whereas coyotes from the northeast zone had the lowest levels of mitochondrial genetic diversity, these same individuals had a level of nuclear genetic diversity comparable to Ohio coyotes. The most pronounced differences between observed and expected heterozygosity occurred in the contact zone and in the overall regional analyses (Table 2.2). When genetic diversity of the five different canid groups is estimated by measuring heterozygosity using all 61,435 SNPs from the canine microarray, dogs appear to be the most genetically diverse. The genome-wide ascertainment bias is toward dogs: the expected heterozygosity of dogs was almost twice that of western coyotes. However, when I measured heterozygosity using only the 16 selected hypervariable SNPs, the ascertainment bias reversed: coyotes appear to be the most genetically diverse, with northeastern coyotes having a very high expected heterozygosity, while dogs appear the least genetically diverse (Table 2.3).

In the region-wide population structure analysis of 247 individuals, the value of K that best explained the data, i.e., the value with the maximum estimated log likelihood from STRUCTURE, is $K = 3$ (Figure 2.2a). This suggests that there are three main genetic

subdivisions in the broad sampling area, but the three groups did not correspond to the three groups inferred by Kays *et al*. (2010) using mtDNA. Although the red cluster in Figure 2.2b includes most of the Ohio coyotes, it is more cosmopolitan, also including many coyotes from the contact and northeast zones. No finer-scale genetic structure was detected in Ohio (N = 16) or in the northeast zone (N = 113), as indicated by $K = 1$ having the greatest explanatory power in those separate analyses (Figure 2.2c, e). In contrast, the STRUCTURE analysis detected ample population genetic structure in the contact zone (N = 118), with $K = 5$ as the most probable number of genetic populations (Figure 2.2d).

Discussion

My results show that coyotes in Ohio are the most genetically diverse in the region when surveyed with nuclear SNPs, as with mtDNA (Kays *et al.* 2010). However, the gradual eastward decay in genetic diversity observed with mtDNA is not replicated with nuclear SNPs. The marked reduction in heterozygosity within the contact zone and in the overall region is likely caused by population structure, i.e., the Wahlund effect. Indeed, analyses of population structure revealed three genetic populations in the overall region, as with mtDNA, but finer levels of structure within the contact zone. However, the three primary populations detected in this study do not correspond to the same subdivisions inferred by Kays *et al.* (2010) with mtDNA. Together, my results suggest that studies based solely on mtDNA should be interpreted cautiously. Discrepancies between patterns observed with mtDNA and nuclear DNA may be caused by true organismal processes, such as male-biased dispersal (Prugnolle & De Meeûs 2002). Alternatively, discrepancies may be caused by marker-specific phenomena such as effective population size, lineage sorting, mutation rate, and coalescent times (Zink &

Barrowclough 2008), or the violation of certain assumptions of mtDNA inheritance, such as recombination, paternal leakage, and heteroplasmy (White *et al.* 2008). Future studies should further evaluate these sources of discrepancies.

Coyotes from Ohio appear to make up a panmictic population, as do coyotes from the northeast zone. The latter result is surprising given the vast geographic area sampled. The failure to uncover more than one genetic population in the northeast zone may be due to the lack of resolution of 16 biallelic loci to detect finer levels of structure. However, it could be that coyotes in the northeast zone are the descendants of a few founders and therefore do not exhibit a strong signal of population genetic structure. A recent genome-wide analysis of population structure in wolf-like canids revealed that coyotes are not well partitioned, except at the broadest continental scale, with northeastern coyotes comprising one subdivision (vonHoldt *et al.* 2011). Unlike Ohio and the northeast zone, the contact zone exhibits a strong signal of population genetic structure. This pattern may reflect the recent merging of two colonization fronts and the highly heterogeneous landscape of western New York and western Pennsylvania.

Our perception of population genetic structure, even in highly vagile organisms where it was least expected, has been refined with steadily improving molecular data and geographic sampling. Initially, no evidence of population structure or isolation by distance was found in coyotes, even at the continental scale, using mtDNA restriction site polymorphisms and nuclear microsatellites (Lehman & Wayne 1991; Roy *et al.* 1994). Various behavioral and historical explanations have been invoked to explain these early genetic patterns. But a more likely explanation is that the patterns of weak differentiation were artifacts of sparse geographic sampling or poor resolution of few molecular markers. More recent studies employing advanced analyses of spatial and genetic data have revealed strong differentiation among parapatric

populations of coyotes and wolves, even in the absence of physical barriers to movement (Sacks *et al.* 2004; Sacks *et al.* 2005; Pilot *et al.* 2006; Musiani *et al.* 2007; Sacks *et al.* 2008). These investigations used multiple loci and dense geographic sampling. However, strong genetic differentiation between adjacent populations of coastal and inland wolf populations in British Columbia was shown with mtDNA sequences (Muñoz-Fuentes *et al.* 2009), demonstrating that fine-scale genetic differentiation can be detected with denser sampling alone, even with one molecular mtDNA marker. My results confirm that finer levels of population structure are detectable in northeastern coyotes. Future studies should focus on the ecological mechanisms underlying this structure, especially because coyotes have only inhabited the region for the last 30-80 years (Parker 1995). Finding ecological correlates of population structure in the absence of obvious physical dispersal barriers would be an interesting example of rapid ecological differentiation.

The whole-genome analysis of variation is dog-biased because SNPs were ascertained from the Dog Genome Project. On the other hand, the hypervariable-marker analysis is coyote-biased because the 16 SNPs were chosen from an ascertainment panel of coyotes. This result highlights the importance of evaluating the ascertainment bias of markers employed in a survey of genetic variation and the necessity of selecting SNPs very carefully to match the question of interest. Although the genotyping success rate of the high-resolution melting curve method was low, this study demonstrates that SNPs derived from the dog genome are a promising tool to address various questions regarding the ecology and evolution of wild *Canis*. For example, in order to better understand the complex hybrid ancestry of northeastern coyotes, species-diagnostic SNPs can be used to quantify the relative contributions of its parental populations. Also, recent advances in the molecular genetics of phenotypic variation in dogs allow the use of

SNPs linked to genes of known function to address long-standing questions about morphological adaptation in northeastern coyotes.

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Table 2.1. Sixteen hypervariable single nucleotide polymorphisms and their primer pairs for high-resolution melt curve genotyping assay. SNP ID corresponds to CanFam2 dog genome assembly chromosomal coordinates. Measures of genetic variability correspond to the initial seventeen northeastern and Ohio coyotes that formed the ascertainment panel. MAF: minor allele frequency; H_O and H_E: observed and expected heterozygosity.

SNP ID	Left primer	Right primer	PCR product size (bp)	MAF	H_O	H_E
chr1:92426160	GGGGTTTCTGAAGTGCTGAC	TGTGATAGCCACAGAAAAGCA	92	0.3824	0.7647	0.4723
chr3:60134962	CACTGAGGAATGCTGGGAAG	TCAGGAAGTCTACTCCAGTGTCTG	80	0.4412	0.7647	0.4931
chr4:33800600	ATCACCTCCAGAAAGCCAAG	TAAGGATCATCCCCTCGTTC	95	0.3824	0.7647	0.4723
chr5:65740765	GGACCTCCATAGGACATCCA	TGTGTGGGGAGATGCAAAT	97	0.5	0.7647	0.5
chr6:17110138	CAGTCACAATGGGGTGTCAG	AAGCGGGAGGTAGTATTACTGGT	97	0.4412	0.7647	0.4931
chr8:69650155	GCTCCTGGCTATTGTATTTTCC	TTCAATTCTGCATGGTTGGT	99	0.4412	0.7647	0.4931
chr10:45343436	TCTTTGAGGACATGGAACGA	TCACTCTGGAGACCAAGACG	94	0.5	0.7647	0.5
chr11:66863044	TGGGTAATTTAATCAACGAGGAA	AAAAGCAAGAGGAGGGAACC	92	0.4412	0.7647	0.4931
chr12:17166054	CAACGGCTGGATTCTGACTA	GCACACTGGTGTAGCAGAGC	118	0.4412	0.7647	0.4931
chr16:9533917	TTGATAAATCAAAACCTGGGATG	GATCTGGCCCACAGCTCA	96	0.4412	0.7647	0.4931
chr17:31508687	CAAAAATCAGGGATACAGACAAG	GCCAGAGAATGCCATCTTTA	100	0.4706	0.8235	0.4983
chr19:50618604	TTTTTCCCTGCCTGATTTTT	TTGGAAAGAGATGTCAAGATGG	92	0.4412	0.7647	0.4931
chr22:57259397	GTAGAGGACACCCTTAGATGTGG	TGTCTGGAGGGAGTTCAACA	95	0.5	0.7647	0.5
chr25:44793770	TGACTCACCCAAGGTGATATG	CAGCTCTGATCATGCCAAAT	100	0.4706	0.8235	0.4983
chr27:5811313	AATCACACACGAGCAACACC	CTGCTTGTCCTGGGATGAA	96	0.4706	0.8235	0.4983
chr37:26421162	GGCTCCCAGCTAACTGTTCA	AGCTATCCAGAAGCCCAAGAG	93	0.4706	0.8235	0.4983

Table 2.2. Genetic diversity measured from mtDNA sequences and sixteen nuclear SNP genotypes. mtDNA data and regional designations from Kays *et al.* (2010). Most individuals genotyped at 16 nuclear SNPs comprise a subset of those individuals sequenced. N: sample size, H_O and H_E: observed and expected heterozygosity.

	mtDNA control region			**Hypervariable SNPs**		
	N	**Haplotype diversity**	**θ (per site)**	**N**	**H_O**	**H_E**
Ohio	30	0.844	0.018	30	0.465	0.435
Contact zone	207	0.721	0.014	177	0.312	0.411
Northeast zone	450	0.664	0.008	178	0.442	0.457
Total	687	0.708	0.013	385	0.388	0.444

Table 2.3. Ascertainment bias of surveying genetic diversity in different groups of canids using SNPs discovered after completion of the boxer genome assembly. 61,435 SNPs from vonHoldt *et al.* (2010; 2011); 16 hypervariable SNPs from this study. N: sample size, H_O and H_E: observed and expected heterozygosity.

		61,435 SNPs		16 hypervariable SNPs	
	N	**H_O**	**H_E**	**H_O**	**H_E**
Northeastern coyotes	14	0.190	0.202	0.763	0.493
Western coyotes	45	0.147	0.182	0.387	0.399
Eastern wolves	19	0.187	0.217	0.278	0.290
Western wolves	32	0.203	0.238	0.271	0.319
Dogs	50	0.234	0.359	0.270	0.387

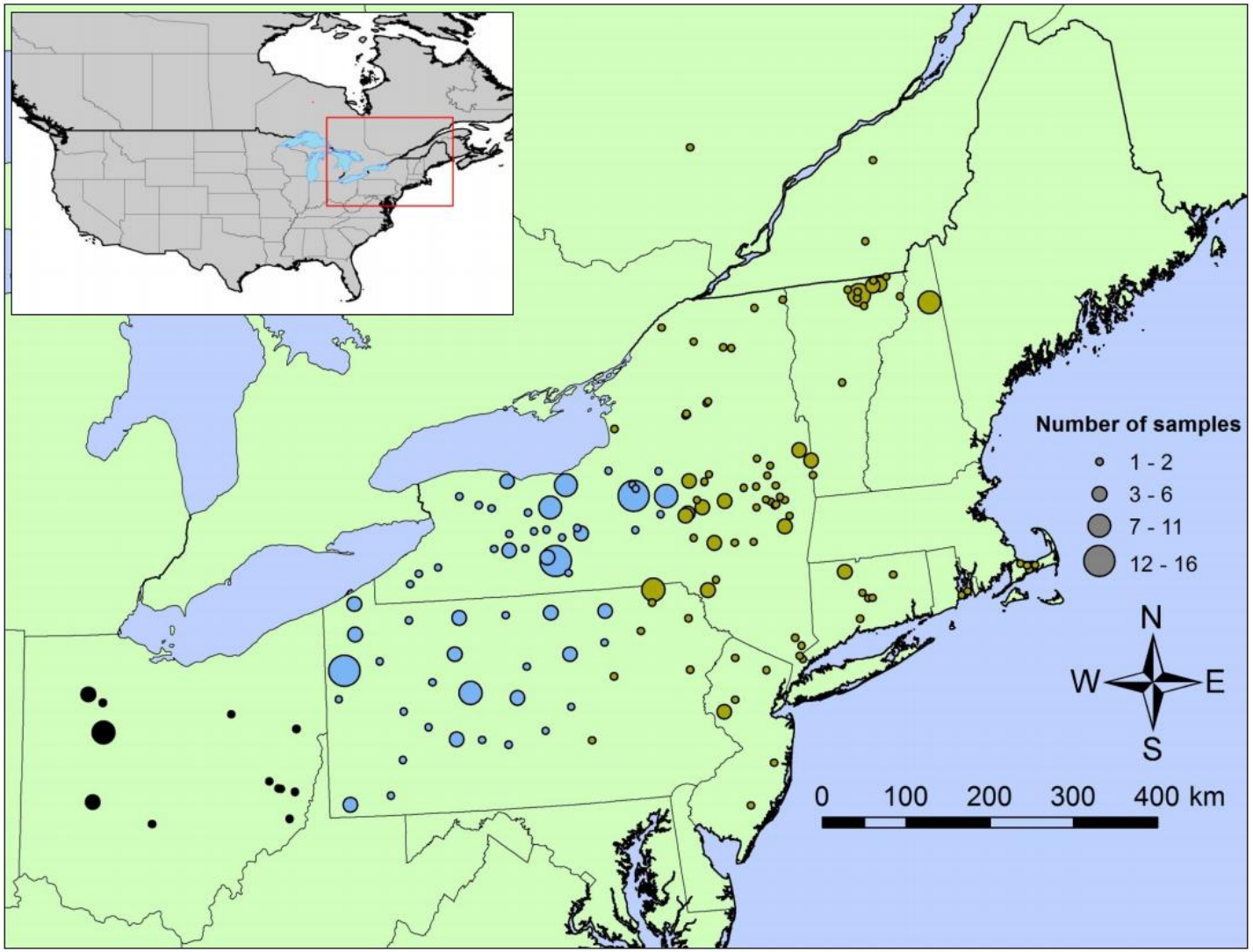

Figure 2.1. Study area and sampling localities of 385 coyotes from northeastern United States and southeastern Canada. Circle size represents sample size per locality. Circle color represents geographic zone as in Kays *et al.* (2010): black, Ohio; blue, contact zone; gold, northeast zone.

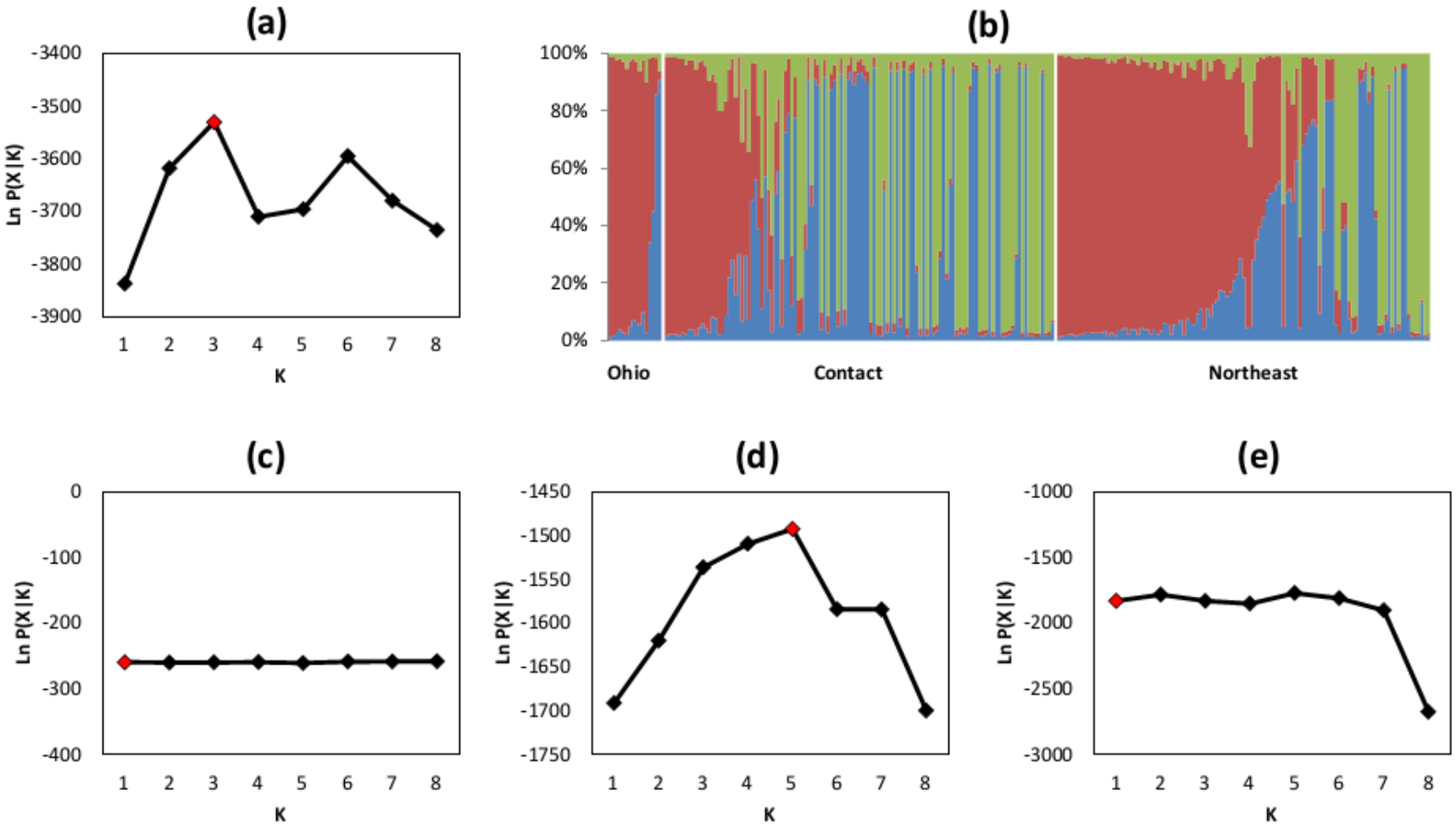

Figure 2.2. STRUCTURE analysis for (a) and (b) N = 247 coyotes across the region, (c) N = 16 coyotes from Ohio, (d) N = 118 coyotes from the contact zone, and (e) N = 113 coyotes from the northeast zone (see Figure 2.1). The most probable number of genetic subdivisions, *K*, i.e., the value with the maximum estimated log-likelihood of the data, in each case is highlighted in red. (b) Bar plot of $K = 3$ in which each individual is represented by a vertical bar partitioned into three segments indicating the admixture proportions or likelihood of assignment to a particular genetic cluster.

CHAPTER 3

ASSESSMENT OF COYOTE-WOLF-DOG ADMIXTURE USING ANCESTRY-INFORMATIVE DIAGNOSTIC SNPS

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J. Monzón, R. Kays, and D. E. Dykhuizen

"It is immaterial for us whether a multitude of doubtful forms be called species or sub-species or varieties…, if the existence of any well-marked varieties be admitted."

- Charles Darwin, *On the Origin of Species* 1859

Abstract

The evolutionary importance of hybridization as a source of new adaptive genetic variation is just beginning to get recognition. Hybridization between coyotes and wolves may have introduced adaptive alleles into the coyote gene pool that facilitated an expansion in their geographic range and dietary niche. On the other hand, hybridization between coyotes and domestic dogs may result in adaptation to human-dominated environments. We genotyped 63 diagnostic single nucleotide polymorphisms in order to examine the prevalence, spatial distribution, and ecology of admixture in eastern coyotes. Using multivariate methods and Bayesian clustering analyses, we estimated the relative contributions of western coyotes, western and eastern wolves, and domestic dogs to the admixed ancestry of Ohio and eastern coyotes. We found that eastern coyotes form an extensive hybrid swarm, and individuals from Ohio, although

slightly more coyote-like, are also highly admixed with wolves and dogs. Coyotes in areas of high deer density are more wolf-like, suggesting that natural selection for wolf-like traits may result in local adaptation at a fine geographic scale. Our results also reveal a pattern of sex-biased hybridization, mediated by male wolves and dogs mating with female coyotes. This study is the most comprehensive genetic survey of admixture in eastern coyotes and demonstrates that the frequency and scope of hybridization can be quantified with a few ancestry-informative diagnostic markers.

Introduction

Hybridization is of immense evolutionary importance as a source of new adaptive genetic variation. Unlike novel mutations, introgressive hybridization simultaneously introduces many alleles that have already passed through the filter of natural selection. Although hybridization has generally been perceived negatively in the conservation and resource management communities (e.g., Rhymer & Simberloff 1996; Allendorf *et al.* 2001; Wolf *et al.* 2001; Oliveira *et al.* 2008), its potential value in enhancing the adaptive potential of parental lineages is gaining recognition (e.g., Kyle *et al.* 2006; Kays *et al.* 2010a). Hybridization has played an important evolutionary role in past range expansions and adaptation to changing environments (Willis *et al.* 2006), and may be vital for the future survival of some taxa under rapidly changing conditions due to anthropogenic land use or climate change. Despite this critical role that hybridization plays in evolution and conservation biology, it remains unclear how habitat variation at the landscape and regional scales affects the flow of introgressed alleles (but see Fitzpatrick & Shaffer 2007).

Modern populations of North American wolf-like canids are known to be admixed in some areas. Coyote-derived DNA was first found in wolf populations of the Great Lakes region in the early 1990s (Lehman *et al.* 1991). A growing body of evidence indicates that the introgressive hybridization among North American *Canis* is very complex, with genetic exchange occurring in varying degrees among western gray wolves (*Canis lupus*), eastern wolves (also known as Great Lakes wolves, *C. lupus lycaon*), Mexican wolves (*C. lupus baileyi*), red wolves (*C. rufus*), coyotes (*C. latrans*), and domestic dogs (*C. familiaris*) (Kyle *et al.* 2006 and references therein; Hailer & Leonard 2008; Kyle *et al.* 2008; Leonard & Wayne 2008; Koblmüller *et al.* 2009; Wheeldon & White 2009; Wilson *et al.* 2009; Bohling & Waits 2011; vonHoldt *et al.* 2011). Although admixture is widely accepted, researchers differ in the interpretations of molecular data and their implications for taxonomic recognition and conservation. Most of the research emphasis has been placed on the wolf side of the admixture story because of ongoing debate regarding the validity of the Great Lakes wolf and red wolf recovery programs, while less attention has been given to the causes and consequences of admixture in eastern coyotes (but see Kays *et al.* 2010a; vonHoldt *et al.* 2011). Hybridization with wolves is believed to have aided coyotes in their colonization of eastern forests by allowing them to rapidly evolve larger body size, including wider skulls, which made them more effective deer hunters (Kays *et al.* 2010a). We hypothesized that individuals living in areas of high deer density are more wolf-like than those living in areas of lower deer density.

Steadily improving molecular tools and geographic sampling have refined our understanding of this hybridization story. For two decades the extent of the molecular data was limited to restriction fragment length polymorphisms (Lehman *et al.* 1991), sequences of mitochondrial DNA (mtDNA) usually coupled with genotypes of a few nuclear microsatellites

(e.g., Wayne & Lehman 1992; Roy *et al.* 1994; Koblmüller *et al.* 2009; Wilson *et al.* 2009; Rutledge *et al.* 2010), and sequences of one gene of the major histocompatibility complex (Hedrick *et al.* 2002). Still, the results of these studies, or more specifically, their interpretations were conflicting. This may be due, in part, to the low resolution offered by analyzing a small number of segregating loci in the context of a complex hybridization scenario. Microsatellites have low statistical power in inferring population structure when samples are drawn from an admixed population (Haasl & Payseur 2010). More recently, vonHoldt *et al*. (2011) published the largest genomic study aimed at addressing the complex evolutionary history of wolf-like canids, taking advantage of the thousands of single nucleotide polymorphisms (SNPs) ascertained from the dog genome project. They used a SNP microarray to assay genomic variation in >48,000 loci genotyped in a panel of 277 wolves and coyotes. Although this was the most extensive genetic survey of any vertebrate group, the geographic sampling of coyotes was limited, with only 13 individuals from northeastern North America and 3 from Ohio, thus limiting inferences about admixture and population subdivision in eastern coyotes.

Here we present data on ancestry-informative SNPs carefully selected and genotyped in a broad geographic sample of 428 eastern coyotes and 2 suspected immigrant wolves, and compare these genotypes to those of 36 western coyotes, 30 western wolves, and 13 eastern wolves from vonHoldt *et al*. (2011). This represents the largest survey of genomic variation in eastern coyotes to date. Our objectives in this study were to use ancestry-informative SNPs to (1) assess the prevalence and spatial distribution of admixture in eastern coyotes, (2) estimate coyote vs. wolf ancestry of individuals, (3) investigate the ecological context of admixture, and (4) test for sex-biased hybridization.

Methods

Study area and sampling

Our study area was located in northeastern North America (Figure 3.1, Table 3S.1). All samples genotyped in this study (N = 427) are archived and vouchered in the New York State Museum, Albany, NY, and were collected with assistance of local hunters and trappers. Two samples (zm14276 from Saratoga County, NY and zm15083 from Orleans County, VT) were suspected wolves based on morphology and preliminary genetics (USFWS 2004; 2007); stable isotope data suggest these two wolves were natural immigrants rather than escaped pets (Kays & Feranec 2011). Thirteen samples came from previous scat surveys in New York (Gompper *et al.* 2006; Kays *et al.* 2008). Three samples from Ohio were genotyped by vonHoldt *et al.* (2011) and were included in our admixture analyses. Because samples came from scat or animals killed for reasons other than research, we did not require IACUC review.

Selection of ancestry-informative SNPs

We selected molecular markers based on a previous study that used the Affymetrix Canine Mapping Array to genotype 60,584 autosomal SNP loci in 57 coyotes, 34 western gray wolves, and 19 eastern wolves (vonHoldt *et al.* 2010; vonHoldt *et al.* 2011). We used two independent but complementary tests to select ancestry-informative SNPs. First, we used the program EIGENSTRAT (Patterson *et al.* 2006; Price *et al.* 2006) to perform a principal components analysis (PCA) of the genetic variance of western coyote, western wolf, and eastern wolf reference populations at all 60,584 loci; we then ranked all SNPs based on their contributions to the first and second principal components. Second, we computed pairwise F_{ST} per locus among the three reference populations and ranked all SNPs based on their degree of

differentiation. We selected SNPs that were present *both* in the top 1% of loci loading the principal component that separates each pair of putative source populations *and* in the top 1% of an analogous F_{ST} comparison (Figure 3S.1).

The goal of our SNP selection process was to come up with a relatively small number of loci with maximum information content to distinguish among three putative parental populations of eastern coyotes: western coyotes, western wolves, and eastern wolves. Although we acknowledge that contemporary eastern wolves are admixed themselves, our approach to selecting SNPs gives the ability to distinguish the relative contributions of eastern and western wolf populations in the genome of eastern coyotes. By choosing SNPs that have a very high F_{ST} and PCA score, we genotyped SNPs whose alleles are not shared by eastern wolves and western coyotes or by eastern wolves and western wolves. Our inclusion of eastern wolves as a reference population does not speak of their taxonomic status. Although they are admixed to begin with, they are still appropriate as a reference group because they are geographically and genetically distinct from the other reference populations (vonHoldt *et al.* 2011) and because admixture with coyotes likely occurred in the Great Lakes region (Kays *et al.* 2010a).

We designed a custom GoldenGate genotyping assay for the Illumina (San Diego, California) BeadXpress platform. GoldenGate is a medium-throughput, PCR-based method of genotyping many loci in one multiplex reaction, and was recently used to survey genetic variation in wild canids (Sacks *et al.* 2009; Sacks *et al.* 2011). We tested in silico the multiplex compatibility of those SNPs that met the above criteria by downloading from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP) at least 60 bp of flanking sequence on each side of the polymorphism and submitting the sequences to Illumina for processing with Illumina's Assay Design Tool (ADT). ADT executes an iterative process that evaluates candidate loci and outputs

an Illumina score for each SNP that could vary from 0 to 1; SNPs with scores >0.7 have a high likelihood of being amplified and genotyped in the multiplex assay. In an initial set of 138 submitted SNPs, the ADT score varied from 0.17 to 0.99. We selected SNPs with Illumina scores >0.7, resulting in a final panel of 63 unlinked SNPs carefully selected to resolve the ancestry of the admixed coyote populations: 21 SNPs diagnostic between western coyote and western wolf, 21 diagnostic between western coyote and eastern wolf, and 21 diagnostic between western wolf and eastern wolf (Table 3.1).

Laboratory methods

We extracted total genomic DNA using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California) according to the manufacturer's instructions or as described in Kays *et al*. (2010a). We cleaned and concentrated genomic DNA using a modified QIAamp DNA Micro Kit (Qiagen) protocol with SpinSmart PCR Purification columns (Denville Scientific, South Plainfield, New Jersey). We determined final DNA concentrations using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, Delaware) and prepared five 96-well plates with genomic DNA aiming to attain uniform concentration (average: 44 ng/μL).

SNP genotyping was done at the Center for Genomics and Human Genetics in The Feinstein Institute for Medical Research, Manhasset, NY. The GoldenGate assay was performed in accordance with manufacturer's protocols. We processed the raw data using the genotyping module of Illumina's GenomeStudio software suite (v2011.1), which automatically generates genotypes from the intensity data. However, for quality control, we removed samples that performed poorly across all SNPs and we removed individual genotypes that did not clearly cluster with the three possible genotype clusters per SNP.

Analyses of admixture

We used two independent approaches to evaluate admixture. First, we used PLINK 1.07 (Purcell *et al.* 2007; http://pngu.mgh.harvard.edu/purcell/plink) to perform multidimensional scaling analysis (MDS). MDS is a data-agnostic multivariate method that allows one to explore and visualize the variation and dominant relationships in genetic data. MDS in PLINK is based on the pairwise identity-by-state distance matrix and the results are comparable to those of PCA. Second, we used STRUCTURE 2.2 (Pritchard *et al.* 2000) to quantify admixture and estimate ancestry in our sample in relation to the three reference populations. STRUCTURE implements a Bayesian clustering algorithm to infer the ancestry of admixed individuals by calculating the posterior mean estimates of *K* proportions of the genome inherited from ancestors in *K* populations. By employing these two complementary approaches, we address the criticisms of each. For example, STRUCTURE and similar Bayesian clustering methods are powerful analytical tools, but assume a population genetics model that may be violated in natural populations. MDS and similar ordination methods simply provide a scatter-plot within which one subjectively looks for interesting patterns, but the data are not required to meet biological assumptions. Therefore, using both complementary approaches strengthens the interpretability of the results (Patterson *et al.* 2006; Rutledge *et al.* 2010).

For the STRUCTURE analysis, we used an initial training set consisting of 40 western coyotes, 34 western wolves, and 17 eastern wolves from vonHoldt *et al.* (2011) as reference parental populations. To ensure that individuals in our training set were "pure" members of their respective groups, we analyzed our parental populations alone at the 63 ancestry-informative SNPs and removed individuals with <95% posterior probability of belonging to the predefined group, following Bohling and Waits (2011). In this analysis we utilized 10,000 burn-in and

100,000 MCMC iterations, used prior population information to assist clustering, used the independent allele frequencies model, and set the number of populations (*K*) to 3. Through this analysis we filtered out 12 individuals, resulting in 36 western coyotes, 30 western wolves, and 13 eastern wolves that formed the final training set to be used to estimate ancestry of the 430 eastern canids. To ensure that the results are not largely affected by our choice of priors, we tested the ability of our 63 ancestry-informative SNPs to distinguish among the three reference populations using all combinations of allele frequency and ancestry models available in STRUCTURE 2.2; we also performed these tests using 63 random SNPs.

We estimated ancestry of each individual in our sample of eastern coyotes using STRUCTURE and the same parameters as described above, following Bohling and Waits (2011). Specifically, for individuals without prior population information (i.e., the "unknown" samples) we used the admixture model and set STRUCTURE to infer α (a Dirichlet parameter for degree of admixture) using a separate α for each population. Even though the ancestry estimate for each unknown individual depends only on the training set and not on the other unknown hybrid individuals, Vähä and Primmer (2006) concluded that STRUCTURE may be somewhat sensitive to the proportion of hybrids in the overall sample. We tested whether the posterior ancestry estimates would be affected by this proportion by analyzing only the Ohio coyotes (N = 26) with the training set (N = 79), and comparing their ancestry estimates with those generated when the full sample (N = 430) is analyzed with the training set.

Although domestic dogs were not included in our SNP ascertainment panel nor in the initial STRUCTURE training set, vonHoldt *et al.* (2011) discovered substantial levels of admixture with dogs, especially in Ohio and eastern coyotes. To test for the degree of dog admixture in our samples of Ohio and eastern coyotes, we conducted a post hoc analysis by

adding a fourth reference population of domestic dogs to our STRUCTURE analysis. For our representative dog population we chose ten modern dog breeds based on their size, potential to mate with coyotes and wolves, and presence in North America (Table 3S.1). We carried out this analysis in STRUCTURE using the same parameters as above, but setting *K* to 4.

In order to investigate the broader ecological context of admixture, we tested our hypothesis that individuals living in areas of high deer density are more wolf-like genetically than those living in areas of lower deer density. We obtained white-tailed deer (*Odocoileus virginianus*) densities from a Quality Deer Management Association 2008 map which depicts deer densities across management units or counties and summarizes data provided by state wildlife agencies (http://www.qdma.com/shop/qdma-white-tailed-deer-density-map). We analyzed the association of wolf ancestry to deer density using a non-parametric Kruskal-Wallis test in R (R Development Core Team 2012). We used the combined western + eastern wolf ancestry estimated when dogs were included in the analysis, thus eliminating the *latrans* and *familiaris* components of individuals' ancestry.

Results

Genotyping results

We attempted to genotype 480 samples at 63 SNP loci. Fifteen samples failed, 36 samples performed poorly, and two samples were mislabeled. Genotyping success did not depend on source of DNA, whether fecal or tissue sample (chi-squared test for independence, $P = 0.142$). Out of 26,901 expected genotypes, 1372 were not called, for a total genotyping call rate of 94.7%. Call rates ranged from 0.83 to 1.0 for loci and from 0.52 to 1.0 for individuals.

Admixture

The distribution of genetic variation among genotyped individuals in relation to the reference populations can be visualized with MDS (Figure 3.2, Figure 3S.2). In this analysis using only the 63 ancestry-informative SNPs, the three reference populations form their own distinct clusters in a manner similar to when the ordination is done on all 60,000+ loci (Figure 3.2, Figure 3S.1). MDS axis 1 clearly separates the two species (*C. lupus vs. C. latrans*), while axis 2 separates the two wolf reference populations (western vs. eastern). Most of the 428 eastern coyote samples form their own cluster that has little overlap with the western coyote cluster. As expected, eastern coyotes are between western coyotes and wolves in MDS-space, with large variability in genomic contributions from each reference population. Most individuals from Ohio are far from western coyotes in MDS-space. All Ohio coyotes are fully within the distribution of contact zone and northeast zone coyotes, even in the first four MDS dimensions (Figure 3.2, Figure 3S.2). The three Ohio coyotes genotyped by vonHoldt *et al.* (2011) were outliers relative to the 23 Ohio coyotes genotyped in this study. In addition, one of the suspected wolf immigrants clearly clusters with western wolves and the other with eastern wolves (Figure 3.2).

We ran STRUCTURE with all 6 model combinations of ancestry and allele frequency priors and the 63 ancestry-informative SNPs performed very well in all simulations (Figure 3S.3a). Thus the "cleanness" of the bar plots in Figure 3S.3a is not a statistical artifact; i.e., the parameters chosen as priors in STRUCTURE's Bayesian approach are not strongly affecting the results. The panel of 63 randomly chosen SNPs did not perform well, except in the usepopinfo model (Figure 3S.3b).

After determining that the 63 ancestry-informative SNPs successfully distinguished among western coyotes, western wolves, and eastern wolves, and that the results are not much affected by modeling decisions, we assessed the ancestry of the 430 eastern canids in relation to the three parental populations. All coyotes genotyped in this study showed a degree of admixture, but there were some geographic differences in the degree of wolf introgression (Figure 3.3, Table 3.2). Ohio coyotes were, on average, 69.45% coyote, and significantly more coyote-like than the contact zone and northeast zone coyotes (Kruskal-Wallis test = 5.6659, P = 0.0173). Coyotes from the northeast zone were significantly more wolf-like than Ohio and contact zone coyotes (Kruskal-Wallis test = 28.2344, $P < 0.001$). When we compared the ancestry estimates of the Ohio coyotes when run alone versus when run with the other 404 coyotes, the differences were negligible, thus the STRUCTURE analyses were not sensitive to the proportion of hybrids in the overall sample. Although the 63 ancestry-informative SNPs were not selected to distinguish dogs from wild *Canis*, they did so remarkably well (Table 3.2). This allowed us to estimate the proportion of dog ancestry in eastern coyotes. There was a consistent signal of dog ancestry, with about 10% of eastern coyotes' genome assigned to dog (Table 3.2). Coyotes sampled from the highest deer density habitats (> 45 deer/mile^2) were significantly more wolf-like (Figure 3.4, Kruskal-Wallis chi-squared = 7.2793, df = 1, P = 0.007).

Discussion

Admixture in North American canids

We analyzed 63 ancestry-informative SNPs in 430 eastern canids and found that admixture is pervasive across the region. The ancestry of all coyotes we sampled showed a clear

signal of hybridization with various *Canis* groups: western wolves, eastern wolves, and domestic dogs. This coyote-wolf-dog hybrid swarm extends into the Midwestern United States. Contrary to our expectations of finding no wolf DNA in Ohio coyotes, these were on average 67-69% coyote and 24-28% wolf, depending on whether dogs are included in the admixture analysis (Table 3.2). The extension of wolf introgression into Ohio was unexpected because vonHoldt *et al*. (2011) found that midwestern/southern coyotes were genetically distinct from hybrid northeastern coyotes, and that admixture in midwestern/southern coyotes was primarily with dogs. In their analyses, midwestern/southern coyotes had, on average, 7.5% dog ancestry and 2.4% wolf ancestry. However, their inference came from a limited sample of 13 northeastern and 19 midwestern/southern coyotes, only three of which were from Ohio. Those three Ohio coyote samples are on the periphery of the statistical distribution of other Ohio coyotes genotyped in this study (Figure 3.2). This was expected since those three coyotes were selected because they were morphologically peculiar.

How did wolf-derived DNA arrive in Ohio? We propose three hypotheses that require further investigation: (1) coyote-wolf hybrids, descendants of the northern expansion front, circled all the way around the Great Lakes and back westward into Ohio; (2) coyote-wolf hybridization occurred in Minnesota or western Ontario (Kays *et al.* 2010b) and the initial colonizers of Ohio were admixed originally; and (3) coyote-wolf hybrids from southern Ontario moved into the southern peninsula of Michigan and then south into Ohio. These three and any other hypotheses must be able to explain the disparate patterns in mitochondrial and nuclear DNA.

During the design phase of our study there was little evidence that hybridization with domestic dogs is prevalent in the Northeast. Way *et al*. (2010) found no dog mtDNA in 67

coyotes from eastern Massachusetts, and Kays *et al*. (2010a) found only one dog mtDNA haplotype in a region-wide sample of 715 eastern coyotes. Consequently, we did not select any SNPs to be diagnostic of dog ancestry. Still, the results of our post hoc analysis of admixture that included dogs are consistent with those of the more recent work of vonHoldt *et al*. (2011). They found that northeastern coyotes have on average 9.1% dog ancestry; we found that region-wide (including Ohio) coyotes have on average 10.6% ± 3.3 (SD) dog ancestry. Together, these results suggest a limited, but appreciable, amount of coyote-dog hybridization in the recent past (11 to 24 generations, estimated by vonHoldt *et al*. 2011). Since then, the dog components of the genome have been diluted and integrated into the wild gene pool through generations of backcrossing with eastern coyotes. The homogeneity of the dog component in wild eastern coyotes and the absence of F1 hybrids suggest there were no recent coyote-dog hybrids in our large sample, but our sampling of wild canids may have missed domestic F1 coyote-dog hybrids (see below).

Our data reveal a complex pattern of admixture among coyotes, dogs, and two distinct wolf populations. We do not believe the common name "Coywolf," proposed for northeastern coyotes by Way *et al*. (2010), captures this complexity. Similar complex patterns of three- and four-way hybridization have been observed in North American *Canis*. Hailer and Leonard (2008) found some degree of hybridization among sympatric coyotes, Mexican wolves, and red wolves in Texas; Bohling and Waits (2011) detected frequent admixture among coyotes, gray wolves, red wolves, and domestic dogs in North Carolina; and Rutledge *et al*. (2010) showed that eastern wolves in Ontario act as a conduit of gene flow between coyotes and western wolves by hybridizing with both. Hybridization in *Canis* extends outside North America: domestic dog

genes have introgressed into the wild Australian dingo, European gray wolf, and Ethiopian wolf (*Canis simensis*) populations (Gottelli *et al.* 1994; Elledge *et al.* 2008; Godinho *et al.* 2011).

Sex-biased hybridization

The observation that dog DNA is present in the nuclear genome but absent in the mitochondrial genome of eastern coyotes reveals that male dogs mated with female coyotes, but not vice versa. Hybridization of European and African wolves with domestic dogs is consistently mediated by male dogs and female wolves (Gottelli *et al.* 1994; Vilà *et al.* 2003; Godinho *et al.* 2011). It is conceivable that our coyote study and the Old World wolf studies failed to sample the hybrid progeny of wild males and domestic females as these pups would likely be reared by bitches in a domestic setting or eliminated by dog owners. In contrast to this general pattern of sex-biased hybridization, Adams *et al.* (2003) documented about 10% of southeastern coyotes with a dog mtDNA sequence, but they postulated a more unnatural cause: young male coyotes from Texas were periodically trapped and released in the Southeast for sport hunting before the main front of coyotes colonized the region; a male coyote that escaped had no female conspecifics and mated with a local dog instead. Our data are consistent with other observations that sexual interactions between wild and domestic canids generally involve male dogs. It is not uncommon for males of certain dog breeds to be as large, or larger, than female wolves or coyotes.

Similarly, the observation that wolf DNA is present in the nuclear genome but absent in the mitochondrial genome of Ohio coyotes is clear evidence of sex-biased hybridization between male wolves and female coyotes. The first genetic evidence of coyote-wolf interbreeding suggested that hybridization is unidirectional and occurs only with male wolves and female

coyotes (Lehman *et al.* 1991). Rutledge *et al*. (2010) also showed that male western wolves tend to cross with female eastern wolves, and that male eastern wolves tend to cross with female coyotes. Interspecific crosses between male coyotes and female wolves are much rarer. Only 1 in 70 Texas coyotes surveyed carried maternal gray wolf DNA (Hailer & Leonard 2008); and although hundreds of eastern coyotes carried maternal eastern wolf DNA (Kays *et al.* 2010a), nearly all carried the same haplotype, suggesting a single hybridization event.

Overall, our data support the hypothesis that the directionality of coyote-wolf-dog sexual interactions is largely determined by body size, with the males of the larger species mating with females of the smaller. But at least three other hypotheses may account for the apparent directionality of hybridization in *Canis*. The relative abundances of coyotes and wolves are very different. Wolf populations, usually being sparse, may be subject to Allee effects. One such effect may be the perception by male wolves of heterospecific females as potential mates. Male wolves may encounter lone female coyotes much more frequently than male coyotes encounter lone female wolves. As mentioned above, strong maternal effects in *Canis* may preclude F1 progeny of domestic mothers and wild fathers to enter the wild population. The body size, Allee effects, and maternal effects hypotheses are not mutually exclusive, but one way to start testing them is to look for coyote and wolf introgression in rural and feral dogs, especially in dogs of different sizes. For example, the size hypothesis predicts greater introgression of wild alleles in litters of smaller female dogs than in those of larger females; and the maternal effects hypothesis predicts the presence of wild *Canis* Y-chromosome diagnostic alleles in domestic male pups, but not in wild male pups.

Ecological context of hybridization

Coyotes living in areas of high deer density are more wolf-like genetically (Figure 3.4), supporting the idea that introgressive hybridization with wolves facilitated the colonization of eastern forests and introduced adaptive genetic variation that allowed coyotes to exploit a prey base rich with ungulates (Kays *et al.* 2010a). However, the association of wolf-likeness with deer density is driven solely by a small sample of 10 individuals from a few localities of very high deer density in New Jersey. The association is not clinal and disappears when the two highest levels of deer density are compared with the two lowest. The local adaptation hypothesis should be confirmed with finer-grained, continuous deer density data and genetic markers linked to traits of known function, such as genes related to body size and skull morphology. Further, an analysis of skulls may reveal that coyotes living in areas of high deer density appear more wolf-like morphologically. Coyote-wolf hybrids in undisturbed landscapes of southeastern Ontario indeed have a more wolf-like morphology and diet, while those in nearby fragmented and disturbed landscapes have a more coyote-like form and diet (Sears *et al.* 2003). These and our results preliminarily indicate that natural selection for wolf-like versus coyote-like traits may be occurring at a fine geographic scale based on landscape characteristics, such as prey availability and human land use.

Methodological matters

Several methodological issues are noteworthy. First, our use of ancestry-informative markers allowed us to quantify the relative genomic contributions of four putative parental populations to eastern coyotes. Fitzpatrick and Shaffer (2004, 2007) employed a similar approach using 8 diagnostic restriction-fragment-length polymorphisms to ascertain the degree

of introgression from an introduced salamander's genes into the gene pool of the threatened California tiger salamander (*Ambystoma californiense*). Talbot *et al*. (2011) likewise developed an assay to diagnose among four poplar (*Populus*) species and their hybrids using 26 diagnostic SNPs. These studies demonstrate how readily hybridization can be quantified with just a few carefully chosen fixed or nearly fixed diagnostic markers. Indeed, a simulation study suggested that 12 loci with an average F_{ST} of 0.21 have sufficient power to detect hybrids of two parental populations, although the hypothetical loci were multiallelic (Vähä & Primmer 2006). The 63 markers we used in this study have very high F_{ST} values (average: 0.79, range: 0.53-0.97; Table 3.1) compared to genome-wide F_{ST} (western coyote-western wolf: 0.14, western coyote-eastern wolf: 0.11, western wolf-eastern wolf: 0.05; vonHoldt *et al.* 2011). As a result we were able to still use the admixed eastern wolves as a reference population in order to assess their relative contribution to eastern coyotes.

Second, several recent studies have employed STRUCTURE or similar Bayesian clustering programs to assess hybridization (e.g., Oliveira *et al.* 2008; Bohling & Waits 2011; Godinho *et al.* 2011; Sacks *et al.* 2011; vonHoldt *et al.* 2011). Most of these studies avoid using prior population information (the usepopinfo ancestry model in STRUCTURE) that may bias the posterior probabilities of assignment. However, the usepopinfo model is necessary when using a sample of reference populations to assess admixture in another population. Pritchard *et al*. caution the user to also run the program without population information to ensure that the pre-defined populations are in rough agreement with the genetic information because this model assumes that the predefined populations are usually correct. We ran our reference samples through the program with and without the usepopinfo model and observed that it is unwise to use prior population information if the genetic markers are polymorphic but not diagnostic. If, on

the other hand, diagnostic markers are chosen to maximize differentiation among groups, user decisions concerning biological assumptions are less influential (Figure 3S.3).

Third, using fecal samples for population-level genetic analyses became popular in the 1990s, but has some technical complications because scat-derived DNA tends to be highly fragmented and degraded (Kohn & Wayne 1997; Kohn *et al.* 1999). The Illumina GoldenGate assay we used worked well to genotype scat samples, most likely because the PCR amplicons are short (100-120 bp). Sacks *et al.* (2011) obtained similar positive results using the same method. Thus, we recommend that researchers working with fecal DNA use a method that amplifies similarly short sequences, such as assays of SNPs and small indels.

Fourth, SNPs are the new vogue in population and conservation genetics (Morin *et al.* 2004; Kohn *et al.* 2006). The completion of the dog genome project enabled researchers to discover and interrogate many SNPs in wild canids. Despite the technological advances, several pioneering studies were fraught with laborious SNP discovery and genotyping methods, small sample sizes, and low genotyping rates (Seddon *et al.* 2005; Andersen *et al.* 2006; Sacks & Louie 2008). As in all molecular studies, researchers must balance the costs of genotyping many individuals versus many loci. We navigated these issues by choosing a mid-throughput genotyping method and judiciously selecting SNPs with the highest information content. This inclined us to genotype many samples versus many SNPs. The resulting dense geographic sampling gives unparalleled resolution to understand admixture dynamics and facilitates future investigations of cryptic population structure (Sacks *et al.* 2004; Sacks *et al.* 2005) and local adaptation.

Philosophical matters and implications for coyote and wolf management

Although hybridization in increasingly accepted as a natural phenomenon, even among vertebrate groups, it is often problematic for conservation practitioners. Many conservation policies have the biological species concept as their foundation, thus assuming that "species" are reproductively isolated. Yet, one million years of divergence from a common ancestor, and tens of thousands of years of intense artificial selection in dogs have been insufficient for reproductive isolation to fully evolve in *Canis*. In many cases, even when reproductive isolation has not fully evolved, there tends to be some outbreeding depression from the loss of locally adaptive genotypes. The opposite outcome, hybrid vigor, appears to be the case in North American *Canis*. In fact, the movement of coyotes into the Northeast did not occur until soon after they began hybridizing with wolves, about 170 years ago (or 86 coyote generations, vonHoldt *et al.* 2011 Supplemental Table S6). A more diverse genome, with genes from both species, likely allowed them to survive in new habitats, both forested and human-dominated. Admixed northeastern coyotes have higher genome-wide heterozygosity than non-admixed populations (vonHoldt *et al.* 2011), and modern admixed Great Lakes wolves have more mitochondrial and Y-chromosome haplotypes than western coyotes and gray wolves (Koblmüller *et al.* 2009).

One phenomenon that is of particular concern is hybridization between a domestic species and its wild relatives. Some examples among vertebrates include bison (Halbert & Derr 2007), wolves (Godinho *et al.* 2011), and wildcats (Oliveira *et al.* 2008). It is worrisome that some wildlife may lose its wildness and untamed nature by hybridizing with a domestic species. Indeed, genetic evidence from a domesticated line of foxes (*Vulpes vulpes*) reveals that domestication leads to marked differences of gene expression in brain regions that modulate

emotions and behavior (Lindberg *et al.* 2005). Introgression of domestic alleles may have made eastern coyotes more adapted to human-dominated environs.

They type of hybridization documented in this study may be perceived in a negative or a positive way. Seehausen *et al.* (2008) noted that hybridization may result in a net loss of species numbers, effectively reversing speciation; they used eastern coyote-wolf hybrids to exemplify how two species may coexist in sympatry in some parts of their range, but merge as a hybrid swarm in another more disturbed area. But they could have also used eastern coyote-wolf hybrids to exemplify the rescuing of local biota or the colonization of a new niche via hybridization. As mentioned above, admixed coyote and wolf populations in the Northeast are more genetically diverse than their parental populations, and this enhanced genetic diversity may be adaptive. In a way, hybridization between coyotes and wolves and the subsequent colonization of eastern forests have yielded a net increase in local species diversity by restoring a large wolf-like canid into the Northeast United States. It is unclear whether this admixed canid will prevent the recolonization of true, full-sized wolves. The two wolves genotyped in this study were likely natural dispersers from Canada (Kays & Feranec 2011). One of them had a genetic profile of western *Canis lupus,* and likely dispersed from northern Quebec where wolves tend to remain free of admixture with coyotes.

Wolf and coyote management policies should consider the ecological importance of large predators. With 16-20 million white-tailed deer in the United States, the direct and indirect socioeconomic costs of overpopulated deer are staggering: annual estimates of deer damage are reported to exceed $2 billion nationwide, including $1 billion/year in car damages (Rondeau & Conrad 2003). There is no question that we need to restore natural predator-prey dynamics, lest we allow deer populations to be regulated by cars. Because eastern North American canids form

a taxonomically complex group characterized by reticulate evolution, we argue that management policies in the region should aim at conserving natural ecological and evolutionary processes (Ennos *et al.* 2005; Kyle *et al.* 2006), such as trophic dynamics and local adaptation.

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Author contributions

JM, RK, and DD conceived and designed the research; JM performed the laboratory work and analyzed the data; DD contributed laboratory materials and reagents; JM wrote the paper.

Table 3.1. Ascertainment and F_{ST} analysis of 63 ancestry-informative SNPs. SNP rs numbers correspond to the CanFam2.0 dog genome assembly. Cla: *Canis latrans*, Clu: *C. lupus*, Cly: *C. lupus lycaon*.

Coyote – Western wolf SNPs				Coyote – Eastern wolf SNPs				Western wolf – Eastern wolf SNPs			
SNP	Cla allele	Clu allele	F_{ST}	SNP	Cla allele	Cly allele	F_{ST}	SNP	Clu allele	Cly allele	F_{ST}
rs24175585	G	A	0.97	rs22927609	G	C	0.96	rs22011433	C	T	0.74
rs22333390	A	G	0.97	rs22416514	G	A	0.95	rs23651611	T	C	0.72
rs9150379	G	A	0.97	rs23054155	G	C	0.94	rs24207725	A	G	0.70
rs22877057	A	C	0.97	rs22691222	C	T	0.92	rs21906101	T	G	0.70
rs24471781	T	G	0.95	rs22659787	C	G	0.90	rs22976400	G	A	0.69
rs23367849	A	G	0.91	rs22436136	A	G	0.90	rs21962387	A	G	0.62
rs24514093	T	C	0.91	rs22491491	G	A	0.90	rs22128776	A	G	0.62
rs24543100	C	T	0.90	rs22488932	C	T	0.90	rs21972855	G	A	0.62
rs23617324	T	C	0.88	rs22494347	T	G	0.89	rs24617980	T	C	0.61
rs22161480	C	T	0.88	rs22582321	C	T	0.87	rs23245491	G	C	0.61
rs8612074	A	G	0.88	rs9073720	C	T	0.87	rs23653965	T	A	0.60
rs23909187	A	G	0.87	rs24489243	G	A	0.86	rs22817050	T	C	0.59
rs23278100	C	T	0.86	rs24373496	G	A	0.85	rs22767921	A	G	0.58
rs23037622	T	C	0.86	rs24447332	C	T	0.85	rs23070823	G	T	0.57
rs22928481	C	A	0.86	rs9029227	G	A	0.85	rs24401025	A	G	0.55
rs23050823	G	A	0.86	rs24218607	T	C	0.85	rs8747831	T	C	0.54
rs23006689	G	A	0.85	rs23126832	T	A	0.84	rs24427396	A	G	0.54
rs24517393	T	C	0.85	rs8666298	G	A	0.84	rs22521423	G	A	0.54
rs22645721	C	T	0.85	rs23410089	G	T	0.83	rs24260906	A	G	0.53
rs22409691	G	A	0.84	rs22350704	C	T	0.83	rs23966574	G	A	0.53
rs23001750	C	A	0.84	rs23486713	G	A	0.83	rs24312148	G	C	0.53

Table 3.2. Ancestry analysis of 430 admixed coyotes in relation to three or four parental populations (in bold): western coyotes, western wolves, eastern wolves, and domestic dogs. Ohio, contact, and northeastern subdivisions correspond to geographic zones in Figure 3.1. Values indicate mean ancestry estimate of N samples.

		Three parental populations			Four parental populations			
	N	**% coyote**	**% western wolf**	**% eastern wolf**	**% coyote**	**% western wolf**	**% eastern wolf**	**% dog**
Western coyotes	36	99.94	0.03	0.03	99.71	0.01	0.01	0.27
Western wolves	30	0.01	99.88	0.11	<0.01	99.85	0.06	0.08
Eastern wolves	13	0.02	0.03	99.95	0.01	0.02	99.83	0.14
Dogs	10	-	-	-	0.01	0.08	0.12	99.79
Ohio coyotes	26	69.45	14.53	16.03	66.84	11.62	12.12	9.41
Contact coyotes	167	68.06	16.17	15.77	65.25	12.83	11.73	10.19
Northeastern coyotes	237	64.22	17.61	18.18	61.30	14.04	13.68	10.98

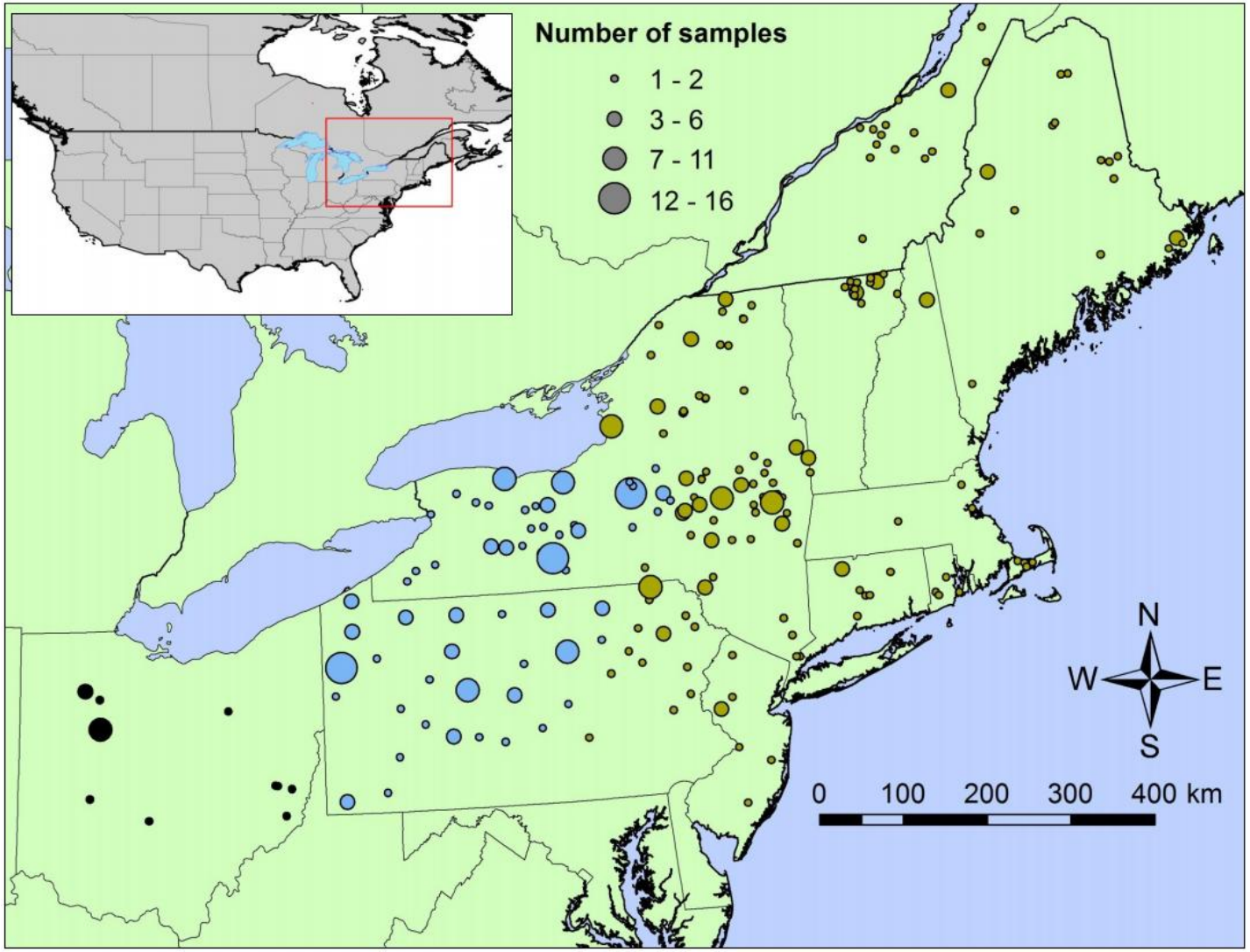


Figure 3.1. Study area and sampling localities of coyotes in northeastern United States and southeastern Canada. Circle size represents sample size per locality. Circle color represents geographic zone as in Kays *et al.* (2010a): black, Ohio; blue, contact zone; gold, northeast zone.

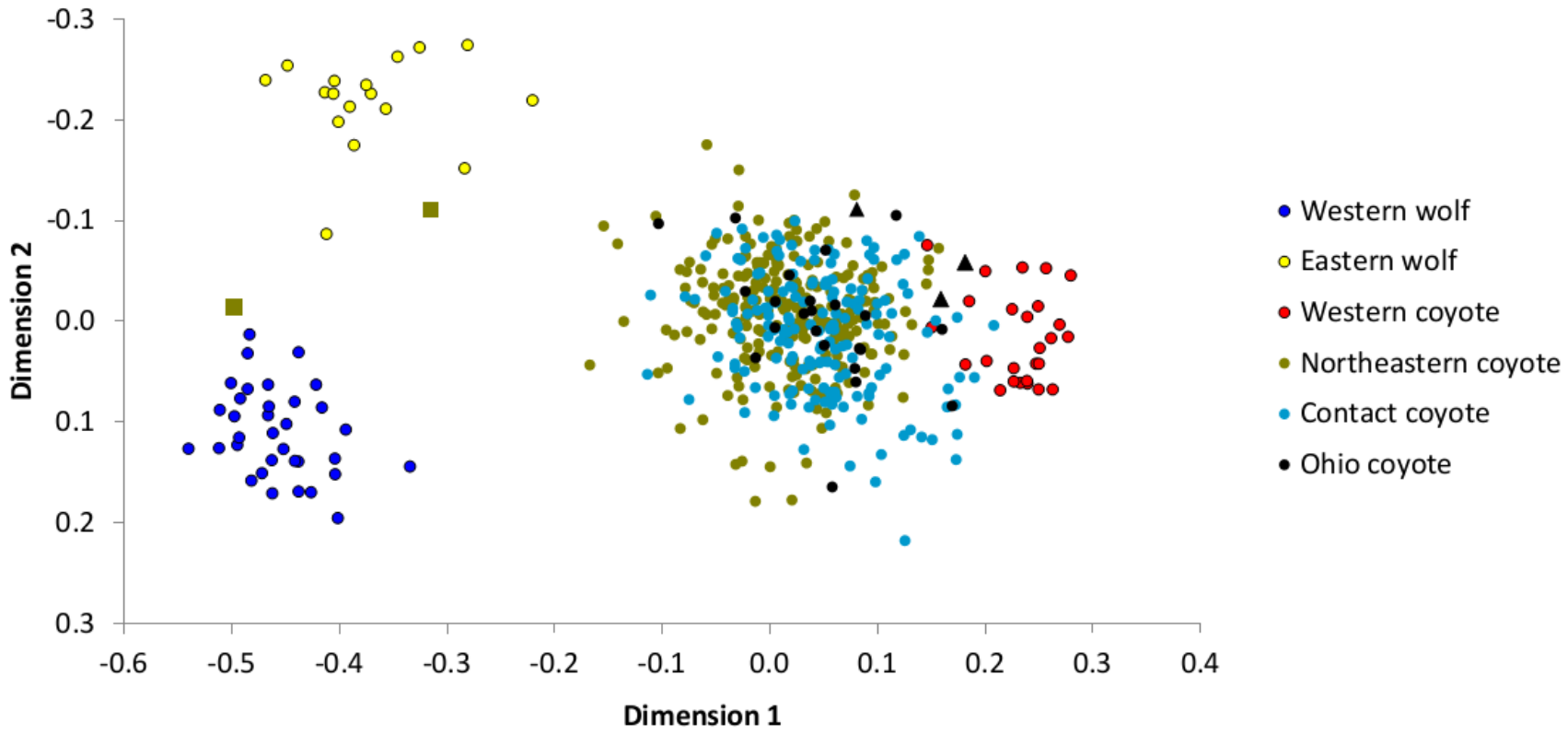


Figure 3.2. Multidimensional scaling plot of the three reference populations and the 427 canids genotyped in this study. Data for all samples are only from 63 ancestry-informative SNPs. Samples genotyped in this study were partitioned into three geographic zones as in Kays *et al.* (2010a): Ohio, contact zone, and northeast zone. Three black triangles represent Ohio coyotes from vonHoldt *et al.* (2011). Two gold squares represent immigrant wolves from New York and Vermont.

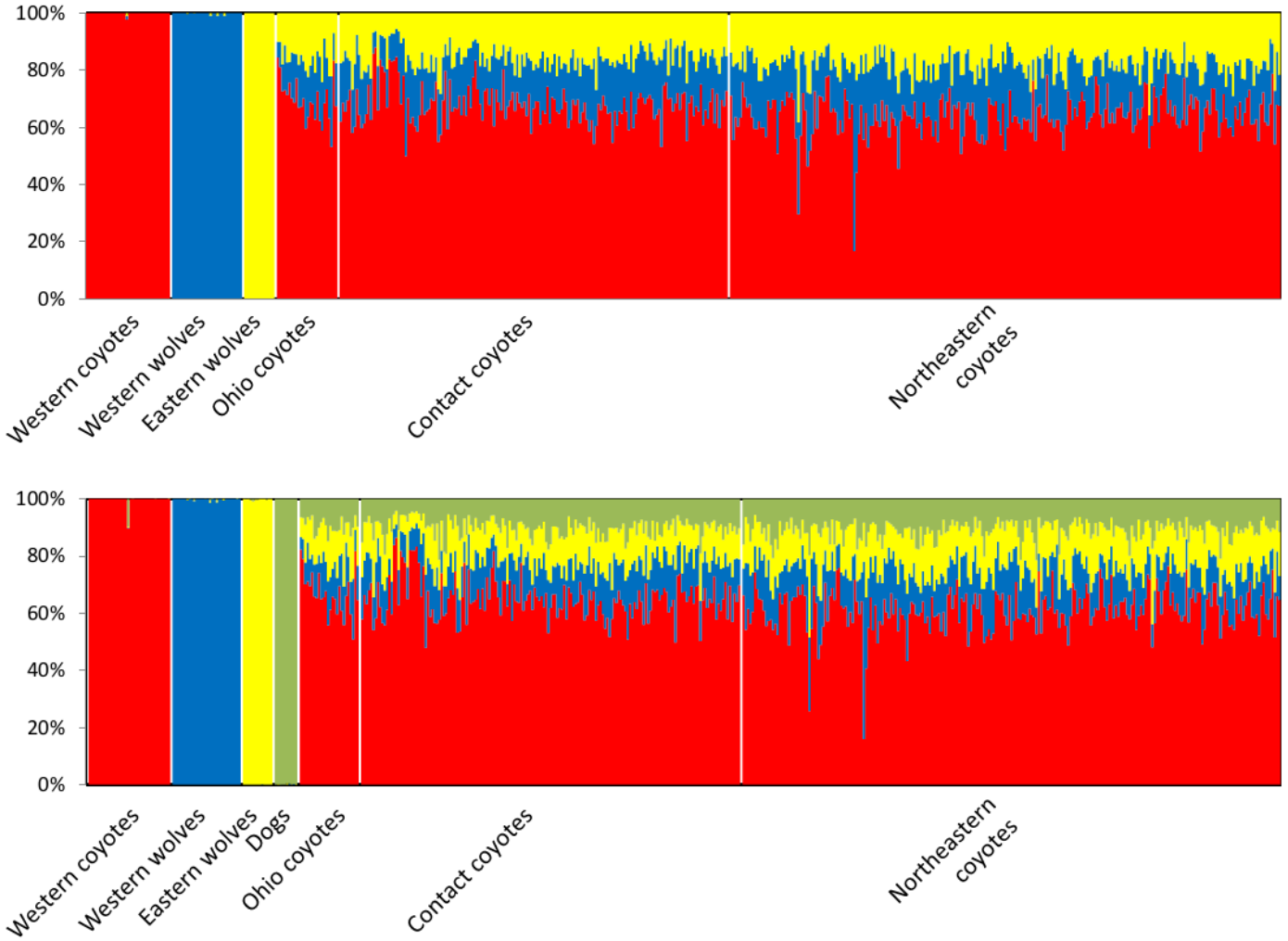


Figure 3.3. Ancestry analyses of 430 admixed canids in relation to the three (top) or four (bottom) parental populations. STRUCTURE bar plots depict each individual as a vertical bar divided into three or four posterior mean estimates of its admixed ancestry, i.e., the estimated proportion of its genome inherited from western coyote, western wolf, eastern wolf, or dog ancestors. Ancestry-informative genetic markers were selected to make the parental populations as distinct as possible. Average ancestry estimates per group are given in Table 3.2.

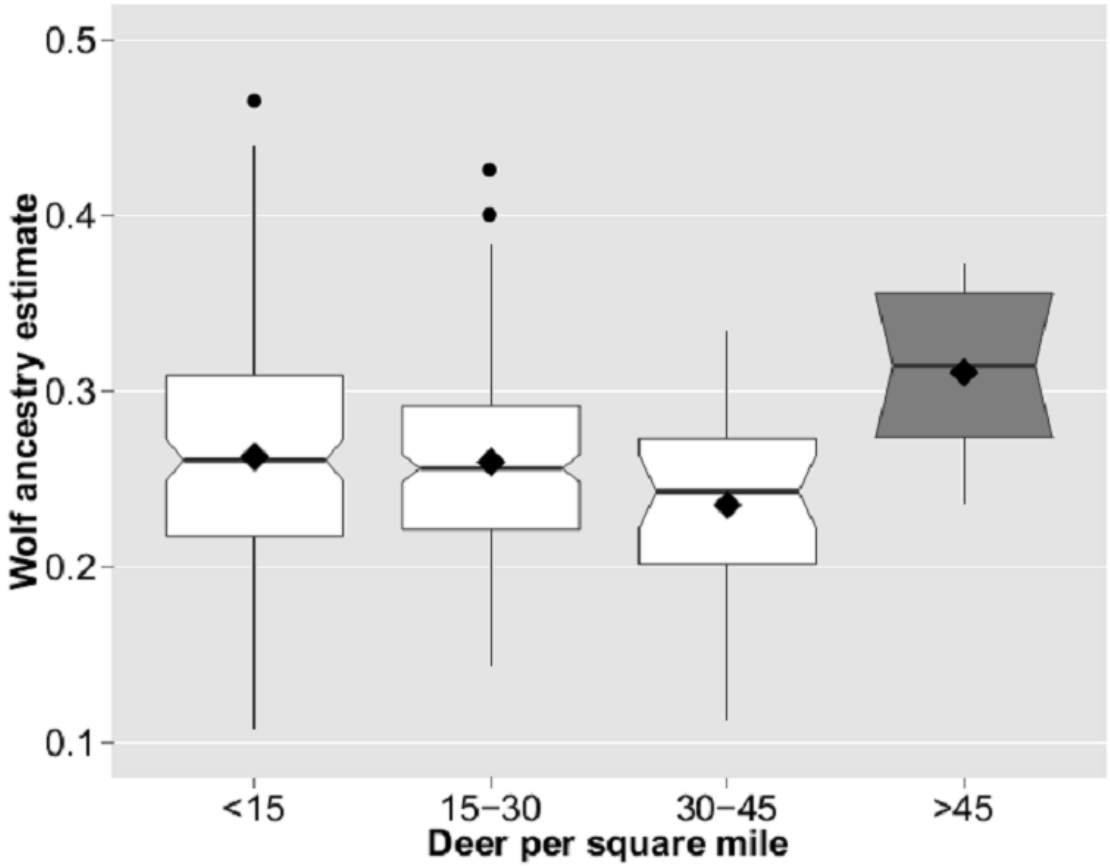


Figure 3.4. Wolf ancestry plotted against deer density. Canids in areas of very high deer density are significantly more wolf-like than in lower density classes, as denoted by the grey box. Wolf ancestry on the vertical axis is the combined western + eastern wolf ancestry proportion estimated when dogs were included in the STRUCTURE analysis, thus eliminating the latrans and familiaris components. The lower and upper boundaries of each box correspond to the first and third quartiles, respectively; black line is the median, black diamond is the mean, circles are outliers. Non-overlapping notches on the sides of boxes indicate strong evidence that the medians differ.

Supporting information

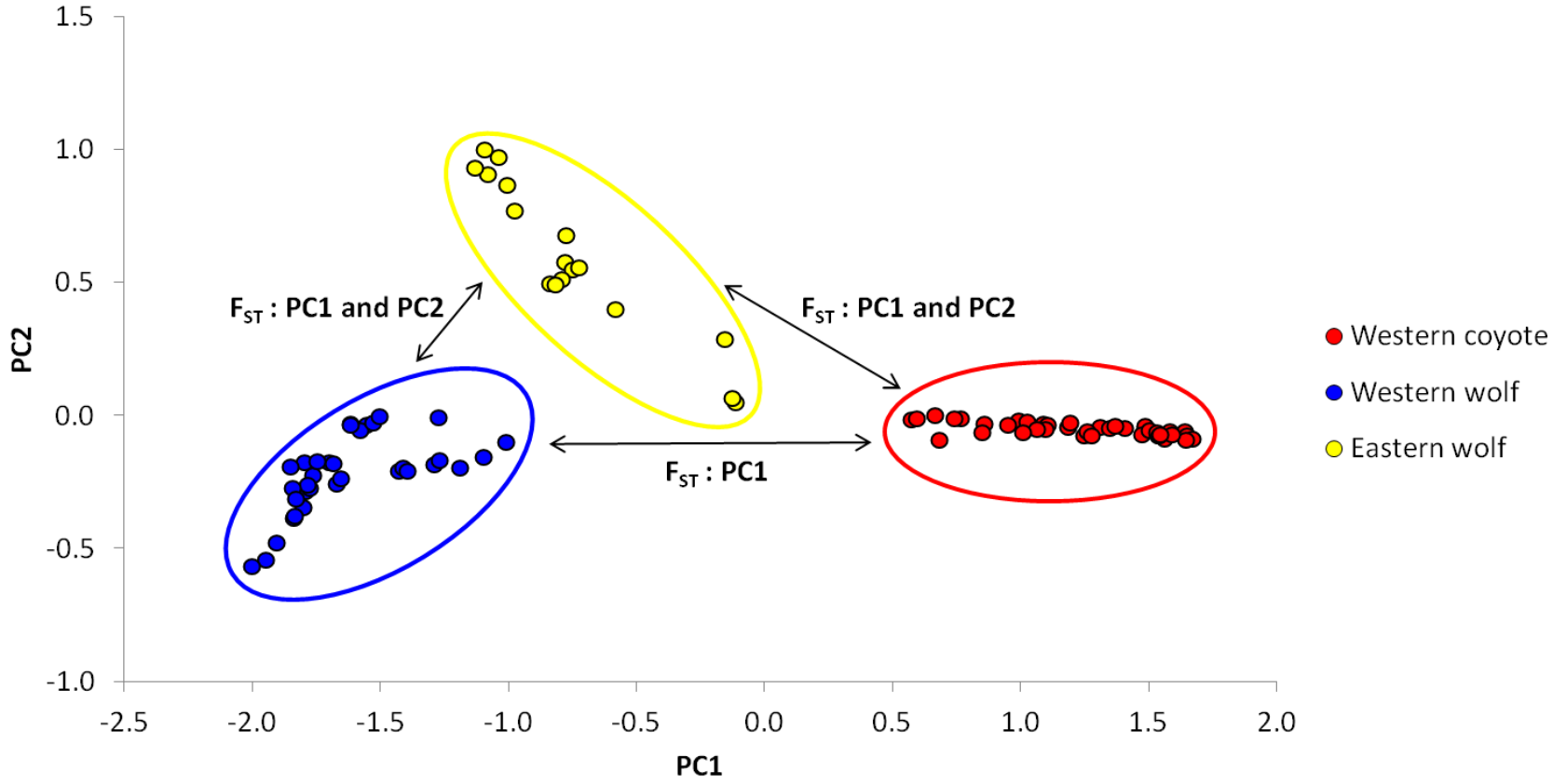


Figure 3S.1. Principal component analysis of initial reference populations of western coyote, western wolf, and eastern wolf genotyped at 60,584 SNPs (vonHoldt *et al.* 2011). Arrow labels indicate how pairwise per-locus F_{ST} estimates were compared to per-locus contributions to the first or second principal component. An initial set of 138 candidate ancestry-informative SNPs was selected because they were present *both* in the top 1% of loci loading the principal component that separates each pair of source populations *and* in the top 1% of an analogous F_{ST} comparison.

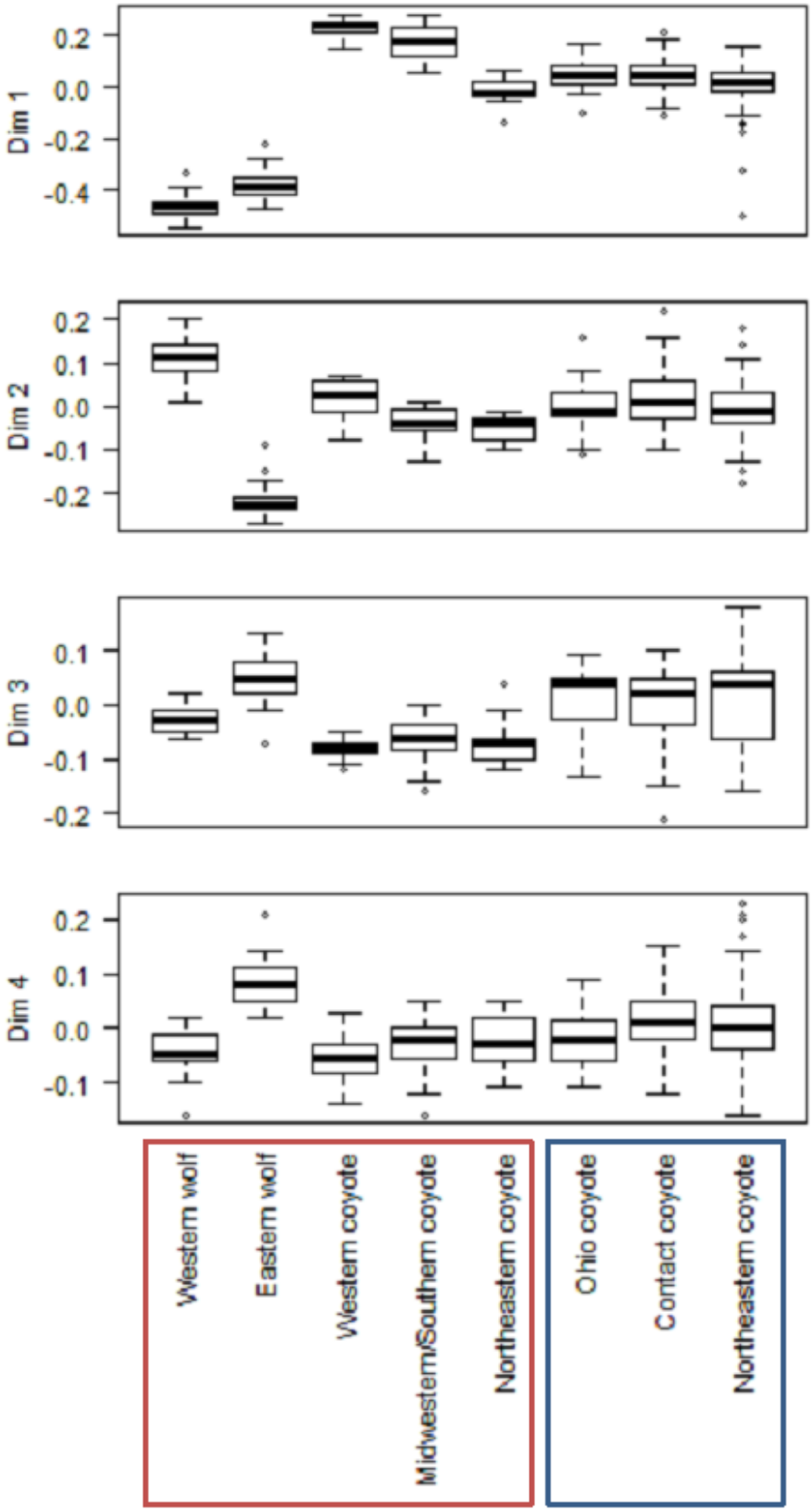
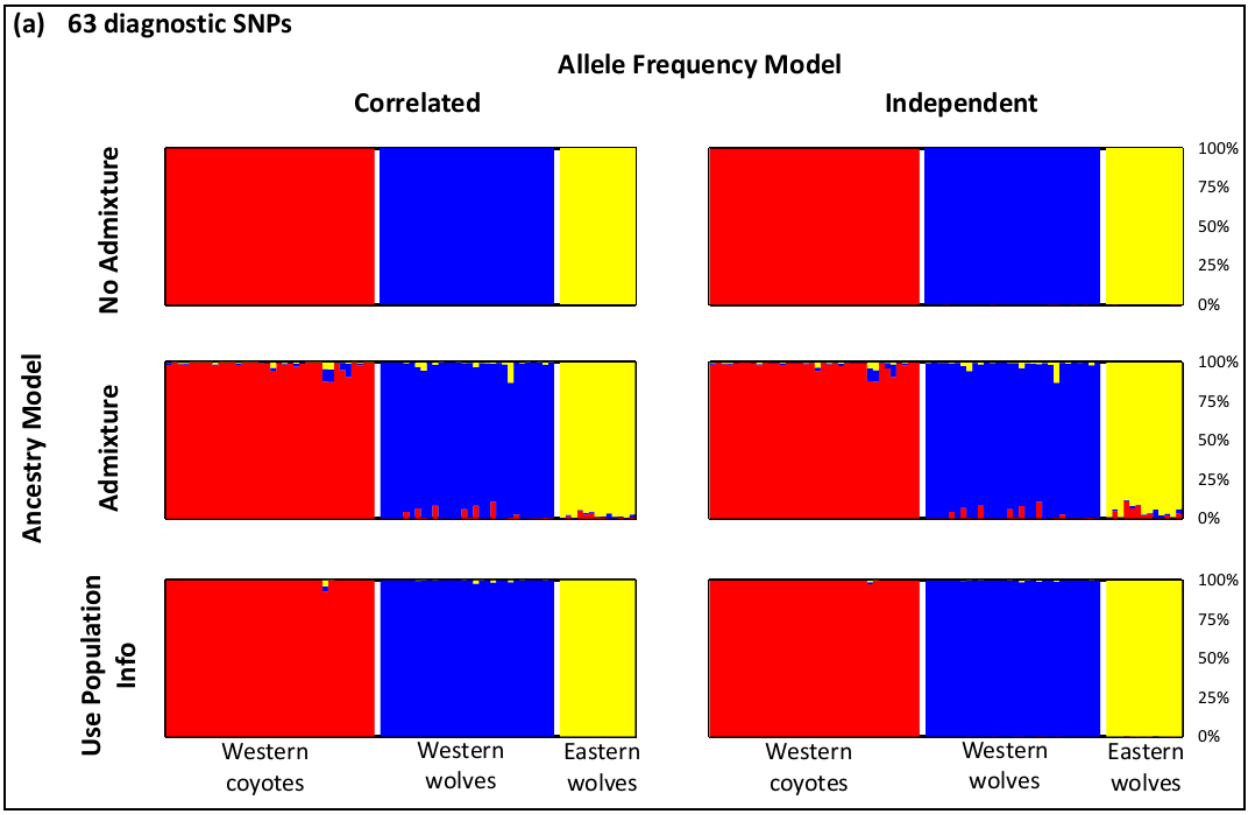


Figure 3S.2. Boxplots of the first four dimensions of MDS. Data for all samples are only from 63 ancestry-informative SNPs. Populations genotyped by vonHoldt *et al.* (2011) in red box, populations genotyped in this study in blue box.


(a) 63 diagnostic SNPs
Allele Frequency Model
Correlated
Independent
Ancestry Model
No Admixture
Admixture
Use Population Info
100%
75%
50%
25%
0%
Western coyotes
Western wolves
Eastern wolves

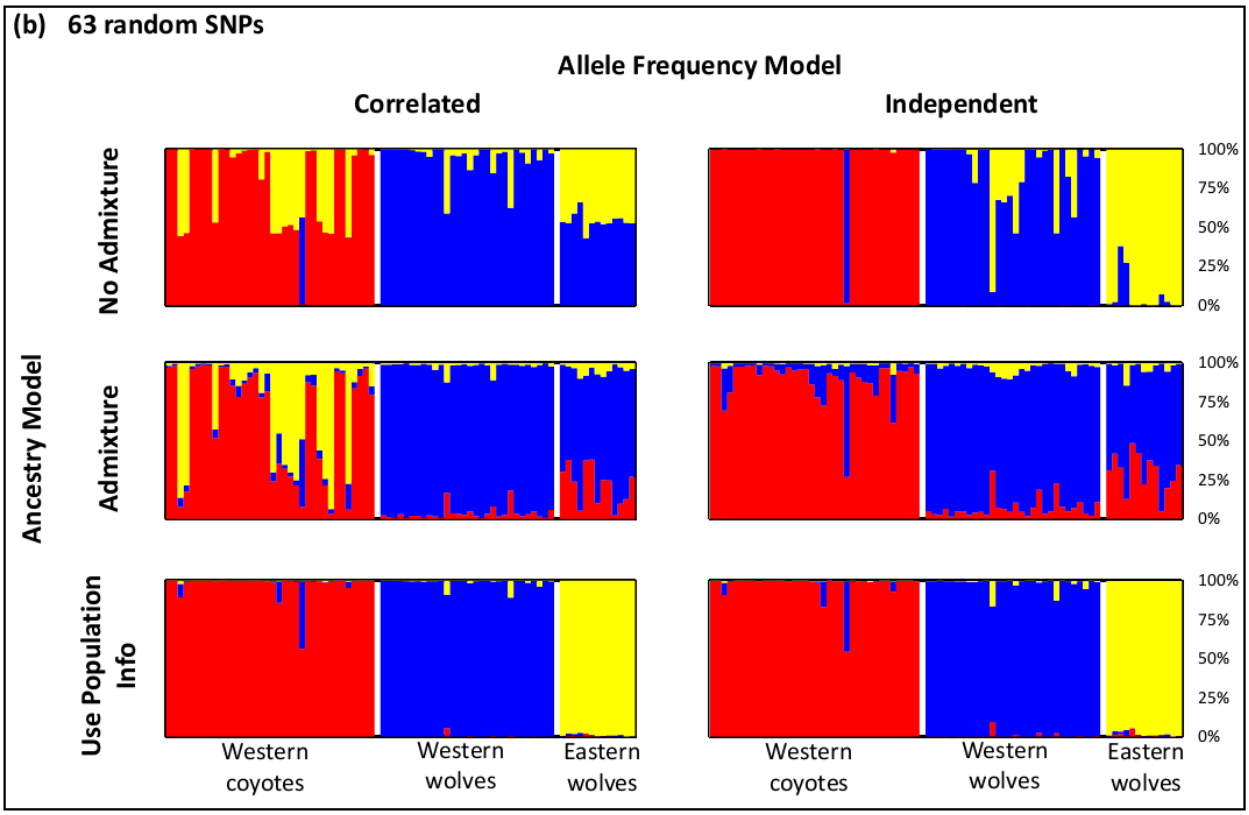

(b) 63 random SNPs
Allele Frequency Model
Correlated
Independent
Ancestry Model
No Admixture
Admixture
Use Population Info
100%
75%
50%
25%
0%
Western coyotes
Western wolves
Eastern wolves

Figure 3S.3. Ability of 63 ancestry-informative SNPs (a) versus 63 random SNPs (b) to distinguish among 36 western coyotes (red), 30 western wolves (blue), and 13 eastern wolves (yellow), using all combinations of the three ancestry models and two allele frequency models available in STRUCTURE 2.2. The purpose of this analysis was to validate the diagnostic capability of our carefully selected SNPs and to test the sensitivity of the results to the priors in STRUCTURE's Bayesian framework. We used the website random.org to generate 63 random numbers between 1 and 48,036 in order to extract the genotypes of 63 random SNPs from the 48K-SNP dataset of vonHoldt *et al.* (2011).

Table 3S.1. Sample sizes (N) of wild and domestic canids whose genotypes at 63 ancestry-informative SNPs were analyzed in this study.

Population	N	Sampling Region* or Breed	Source
Western wolf	34	Alaska (4), Western Canada (15), Northern Quebec (6), Wyoming (7), Ontario† (1), Minnesota† (1)	vonHoldt *et al*. (2011)
Eastern/Great Lakes wolf	17	Ontario (3), Minnesota (10), Wisconsin (4)	vonHoldt *et al*. (2011)
Coyote	54	Alaska (2), California (12), Manitoba (5), Utah (3), Washington (4), Alabama (2), Illinois (5), Louisiana (3), Mississippi (2), Ohio (3), Virginia (4), New York (5), Vermont (2), New Hampshire (1), Southern Quebec (1)	vonHoldt *et al*. (2011)
Dog	10	Rottweiler, Australian Shepherd, Border Collie, Golden Retriever, Labrador Retriever, Giant Schnauzer, German Shepherd, Old English Sheepdog, Doberman Pinscher, Collie	vonHoldt *et al*. (2011)
Ohio coyote	23	Ohio	Monzon *et al*. (this study)
Contact zone coyote	167	New York (79), Pennsylvania (88)	Monzon *et al*. (this study)
Northeast zone coyote	237	Maine (20), Massachusetts (7), New Hampshire (5), New Jersey (14), New York (110), Pennsylvania (25), Southern Quebec (21), Rhode Island (4), Vermont (24), Connecticut (7)	Monzon *et al*. (this study)

* Sample size per region indicated in parentheses

† Two wolves from the Great Lakes region had genetic profiles of western wolves, as determined from principal component analyses

CHAPTER 4

HUMAN LAND USE AND PREY DENSITIES AFFECT THE RAPID FORMATION OF METAPOPULATION GENETIC STRUCTURE IN NORTHEASTERN COYOTES

"On separate continents, and on different parts of the same continent when divided by barriers of any kind, and on outlying islands, what a multitude of forms exist, which some experienced naturalists rank as varieties, others as geographical races or sub-species, and others as distinct, though closely allied species!"

- Charles Darwin, *On the Origin of Species* 1866

Abstract

How populations of mobile species differentiate genetically and specialize ecologically, despite the absence of physical barriers, is of great interest. Recent studies have highlighted the potential for ecological factors to drive genetic structure in animals using landscapes with relatively simple habitat configurations in which the different habitat types are broad and individually continuous. I investigated whether ecological factors can similarly influence genetic structure in a complex, fine-grained mosaic of habitat types. I used an array of 91 single nucleotide polymorphisms to test the relative importance of geographical, ecological, and historical factors to explain genetic variation in a sample of 427 northeastern coyotes (*Canis latrans*), a species that colonized the region in the last 70 years. I found limited evidence for isolation by distance but did detect a pattern of subtle population genetic structure. Bayesian and spatial autocorrelation analyses supported the hypothesis of a metapopulation structure

conforming to patterns of forested, agricultural, and urban habitat types. High densities of white-tailed deer (*Odocoileus virginianus*) also explained a small, but significant, proportion of genetic variation. Given the recency of the coyote range expansion into the Northeast, these findings demonstrate the rapid formation of ecological barriers to gene flow in just a few dozen generations. I suggest the induction of strong habitat preferences early in life as the underlying mechanism for the observed pattern of habitat-conforming genetic metapopulations.

Introduction

A burgeoning literature is revealing that there can be substantial genetic differentiation among neighboring populations of widely distributed and highly mobile species. It is surprising that adjacent populations can accumulate genetic differences despite the apparent absence of obvious barriers to movement and high intrinsic potential for gene flow. The mechanisms driving these differences are of great interest because they directly relate to incipient speciation and the origin and maintenance of biodiversity (Sacks *et al.* 2008; White *et al.* 2011). A primary focus has been on establishing ecological explanations to account for such genetic divergence between parapatric populations (Doebeli & Dieckmann 2003; Schluter & Conte 2009) or even sympatric individuals (Bolnick *et al.* 2003). For example, extensive genetic differentiation has been observed in adjacent populations of many large mammals, including carnivores (Rueness *et al.* 2003a; 2003b; Sacks *et al.* 2004; Pilot *et al.* 2006; Musiani *et al.* 2007; Muñoz-Fuentes *et al.* 2009), ungulates (Courtois *et al.* 2003; Brown *et al.* 2007; Pease *et al.* 2009), and cetaceans (Hoelzel *et al.* 1998a; 1998b). In all these examples, genetic subdivisions were associated with ecological factors and/or local foraging specializations.

The surprising patterns of cryptic genetic structure among parapatric populations without

obvious physical barriers have been documented mostly in landscapes with relatively simple habitat configurations, in which the different habitat types are geographically broad and individually continuous. For example, Musiani *et al.* (2007) found marked differentiation between tundra and boreal forest wolves (*Canis lupus*) in northern Canada, and Sacks *et al.* (2004; 2005; 2008) detailed how genetic subdivisions in coyotes (*Canis latrans*) correspond with discrete bioregions in California despite the lack of physical barriers to movement. In all cases, genetic differentiation was not explained by any topographic barriers, but was better explained by habitat specialization, suggesting that ecological factors drive genetic differentiation even in highly vagile species.

What if habitat specialization occurred in a landscape where the different habitat types are not continuous but instead are distributed in a complex mosaic? Natal experience may bias habitat preferences, such that an individual disperser bypasses unfamiliar habitats to settle in a familiar one (Davis & Stamps 2004). For instance, in a mosaic of urbanized, rural, and forested habitats, urban-raised individuals may disperse to other distant urban areas, forest-raised individuals may disperse to other distant forested areas, and so on. In this case, we might expect to observe a collection of genetically differentiated and overlapping metapopulations in which the habitat of one specialist is the matrix of another. The resulting spatial-genetic pattern should be one of various patches of local autocorrelation and little global autocorrelation, except at the shortest distances. If the landscape was recently colonized, as in a rapid range expansion, not enough time may have elapsed for the expected genetic pattern to develop, unless individuals' habitat and assortative mating preferences are strong.

The northeastern coyote provides an ideal system to address these questions. Coyotes are recent colonizers of northeastern North America's forests (Kays *et al.* 2008), croplands (Person

& Hirth 1991), suburbs (Gompper 2002b), and cities (Way 2007). Although they are collectively a generalist species distributed continuously across a complex mosaic of habitat types and human land use types, individuals have strong habitat biases (Sacks *et al.* 2008). This ecological variability occurs at the scale of coyotes' dispersal distances (averaging 102 km, Harrison 1992), making it a good system in which to test hypotheses about how geographical and ecological factors may induce genetic differentiation. Additionally, varying levels of admixture with wolves and dogs (*Canis familiaris*) in eastern North America may influence how individuals utilize the landscape. Sears et al. (2003) studied coyote-wolf hybrids in southeastern Ontario and found that individuals inhabiting undisturbed forests had more wolf-like body morphology and diet, whereas those inhabiting more disturbed and less forested habitats tended to be more coyote-like. Moreover, I found that coyotes in areas of high deer density are genetically more wolf-like (Chapter 3). Though this issue of hybridization has received considerable attention in eastern wolves, only one course-grained study has examined the relationship between hybridization and population genetic structure in northeastern coyotes (Kays *et al.* 2010).

The objective of this study was to examine the structure of genetic variability and its underlying mechanisms in a highly mobile and generalist carnivore. I adopted a fine-grained landscape genomics approach to test hypotheses about the influences of geography, habitat variability, and admixture on the population genetic structure of northeastern coyotes. Specifically, I investigated the extent that ecological factors and hybridization explain the partitioning of genetic variation in northeastern coyotes. The null hypothesis is that there is no spatial genetic structure in the region because individuals comprise one large panmictic population. A simple alternative hypothesis is that genetic structure reflects a pattern of isolation

by distance, with an eastward or southward cline of decreasing variation reflecting the colonization of the region by a few founders from Ohio and southern Canada. However, ecological and recent historical factors may play a role in structuring genetic variation beyond what is expected by geographical distance alone. Therefore I also tested hypotheses concerning the potential influences of anthropogenic land use, prey density, and hybridization with wolves on genetic differentiation. My results demonstrate the potential of ecological variation occurring in a complex, human-modified mosaic to rapidly induce genetic differences in few generations.

Methods

Study area and sampling

The study area and specimens I used in this analysis of population genetic structure were described in Chapter 3. Briefly, 427 samples were collected from hunters, trappers, and scat surveys in Ohio, Northeast United States, and southern Quebec between 1999 and 2009. For spatial analyses, I excluded 21 specimens from Quebec because no land cover and prey density data were available in comparable resolution to the United States. I also excluded two specimens because of their unacceptably large spatial uncertainty (i.e., their most precise locality description was the state: New Jersey and Connecticut) and five other specimens with irreconcilable inconsistencies that precluded accurate georeferencing. I performed all spatial analyses on the remaining 399 canids, which included two large, wolf-like individuals with an estimated coyote ancestry of 17% and 30% (Chapter 3).

Selection of hypervariable SNPs

I selected 91 hypervariable SNPs to genotype in northeastern coyotes. Sixty-three of these SNPs were the same as described in Chapter 3. Average observed heterozygosity of the 63 SNPs in an ascertainment panel of 15 eastern coyotes was 0.47. From the 61,435 SNPs genotyped by vonHoldt *et al.* (2011), I selected an additional set of 28 unlinked, autosomal SNPs with very high variability (observed heterozygosity ≥ 0.8) in the same ascertainment panel of 15 eastern coyotes. The assay design, *in silico* testing of candidate SNPs, laboratory methods, and processing of raw data for quality control are also described in Chapter 3.

Descriptive and spatial statistics

For non-spatial analyses of 427 samples, I calculated expected and observed heterozygosity and tested for Hardy-Weinberg equilibrium at each locus. I calculated a pairwise, individual-by-individual matrix of squared genetic distances. In order to explore and visualize the variation and dominant patterns in the multi-locus genetic data, I performed a principal coordinates analysis (PCoA) based on the conversion of the distance matrix to a covariance matrix.

For spatial analyses of 399 samples, I converted Lat/Long coordinates into UTM coordinates using the PBSmapping package for R (R Core Team 2012) because planar coordinates, such as UTM, are more appropriate for calculating distances than spherical coordinates. I used a Mantel test to examine the correlation between linear genetic distance and geographic distance, using 999 random permutations of the genetic distance matrix to test the significance of the correlation.

I analyzed global patterns of spatial autocorrelation by computing a correlation in each of thirty 50-km distance classes, encompassing the whole study area. The autocorrelation coefficient describes the genetic similarity between pairs of individuals within the specified distance class. Following Peakall *et al.* (2003) and Double *et al.* (2005), I tested the statistical significance of the autocorrelation at each distance class using two methods: 999 random permutations to define the 95% confidence interval of the null hypothesis of $r_G = 0$, and 999 bootstraps to define the 95% confidence interval of the observed autocorrelation coefficient. Furthermore, I tested the overall significance of the autocorrelogram using the nonparametric heterogeneity test of Smouse *et al.* (2008).

To examine the pattern of genetic structure more closely, I used the method of Double *et al.* (2005) which permits the analysis of local spatial autocorrelation. This analysis focuses on a subset of nearest neighbors surrounding a pivotal individual. For each such subset, I computed r_L, the local autocorrelation, and used 9999 permutations to test the null hypothesis of no local structure. Because neighboring estimates of r_L share some overlapping samples and are themselves correlated, I used a Bonferroni-type adjustment so that significance of the permutation test is declared when $P < 0.001$. I applied the conditional permutation approach, in which the pivotal individual is held fixed, while the remaining samples are shuffled over all other locations in the dataset (Double *et al.* 2005). I conducted five runs of the local spatial autocorrelation analysis, with 4, 8, 12, 16, and 20 nearest neighbors. I conducted all analyses described in this section in GenAlEx 6.5 (Peakall & Smouse 2006; 2012).

Bayesian inference of spatial genetic structure

In order to infer the number of genetic subdivisions, I used the Geneland 4.0.3 (Guillot *et al.* 2005a; 2005b) package for R (2012). Geneland implements a Bayesian clustering algorithm to infer *K*, the number of genetic populations, and assigns multilocus genotypes to genetic clusters by calculating the likelihood that a group of individuals constitutes a population in Hardy-Weinberg and linkage equilibria. Additionally, Geneland explicitly incorporates the spatial coordinates of sampled individuals and allows for uncertainty in these coordinates. Although the Bayesian algorithm implemented in Geneland is informed by spatial data, the output is not constrained to produce genetic subdivisions that are spatially continuous. This makes Geneland suitable to identify disjunct patches that may constitute a genetic metapopulation. I used the correlated allele frequencies model and ran the Markov chain Monte Carlo procedure two times to verify consistency of results with the following parameters: 500,000 iterations with a thinning of 500, minimum *K* set to 1, maximum *K* set to 20, uncertainty of spatial coordinates set to 5 km, maximum rate of Poisson process set to 100, maximum number of nuclei in the Poisson-Voronoi tessellation set to 399. I calculated the posterior density of the model using a burn-in of 100,000 iterations, and the posterior probability of population membership for each individual and each pixel using a burn-in of 100,000 iterations and a discretization of the study domain into 260 horizontal and 180 vertical pixels. I used ArcMap 10 to import and digitize the Geneland maps. I used GenAlEx 6.5 to compute F_{ST} among the populations inferred by Geneland, and to test the significance of the structure inferred by Geneland by analysis of molecular variance (AMOVA) using 999 permutations.

Associations of genetic variation with geography, ecology, and hybridization

To investigate the extent that genetic variation among coyotes is explained by geographical distance, ecological factors, and hybridization I conducted a distance-based redundancy analysis (Legendre & Anderson 1999; McArdle & Anderson 2001; Legendre & Fortin 2010). This analysis is a form of nonparametric multivariate multiple regression that can be used to assess the associations of several predictor variables and a dissimilarity response matrix. The response matrix of interest was a matrix of pairwise, individual-by-individual, linear genetic distances, calculated in GenAlEx. The predictor variables were assembled into four sets (Table 4.1). Latitude and longitude together describe geographical distances among individuals. Anthropogenic land use was classified as four types: forest, agricultural, urban/suburban, and open (natural grassland, shrubland, etc.). These four land use types are sufficiently different in several aspects relevant to coyote ecology, such as prey types and abundances, canopy cover, artificial food availability from anthropogenic waste, and human hunting and disturbance. I obtained land cover data in 30-m × 30-m resolution from the US Geological Survey Land Cover 2001 Database (http://www.mrlc.gov/nlcd2001.php) and extracted the data in two different ways, depending on the precision of a sample's georeferenced coordinates. For 140 individuals whose spatial information was known at a finer scale than the county (e.g., township, specific locality, or GPS coordinates), the proportions of the various land use classes were extracted from circular buffers around the georeferenced coordinates. To determine the radius of each buffer, I calculated uncertainty radii for their georeferenced coordinates using the GEOLocate Web Application (http://www.museum.tulane.edu/geolocate) and added 5 km because coyotes are motile and sample the landscape within their home range. Assuming circular home ranges, a 5-km radius includes almost all observed home ranges for eastern coyotes (Chapter 1, Table 1.1).

The average buffer radius was 8.9 km (range: 6.0-19.5 km). For the other individuals who had coarser spatial information, I extracted land cover from the county polygons plus 5-km buffers. This way each sample was associated with land cover at the most relevant spatial scale and the different sources of spatial uncertainty remained additive. Density of white-tailed deer (*Odocoileus virginianus*) was another categorical variable, available in four density bins from Quality Deer Management Association (http://www.qdma.com/shop/qdma-white-tailed-deer-density-map). Deer density provides a proxy for food availability because deer comprise a major portion of northeastern coyote diet (Gompper 2002a). Lastly, hybridization was a binary variable with one and zero indicating the presence or absence, respectively, of a wolf mitochondrial haplotype. I included hybridization as a variable to test the association of the mitochondrial and nuclear genomes. I obtained mitochondrial haplotypes from Kays *et al.* (2010).

Following Anderson *et al.* (2004) and Musiani *et al.* (2007), I conducted marginal tests analyzing the relationship between the response matrix and each predictor variable or set of variables individually, ignoring all others. Next, I subjected the individual variables or sets of variables to a stepwise forward-selection procedure, which consists of sequential tests, fitting each variable or set of variables one by one, conditional on the variables already included in the model. The forward-selection procedure allowed me to control for multicollinearity among predictor variables. I used the software DISTLM forward 1.3 (Anderson 2003) for all multivariate analyses described in this section. For both marginal and sequential tests, *P*-values were obtained using 9999 permutations.

Results

Descriptive and spatial statistics

For the complete set of 427 northeastern canids, there was a general deficiency of heterozygotes, relative to expected proportions (Table 4.2). All 91 SNPs were polymorphic in the strict sense, but seven loci had a minor allele frequency of less than 5%. Thirty percent of loci were not in Hardy-Weinberg equilibrium. The principal coordinates analysis revealed a complex pattern of genetic variation in northeastern canids (Figure 4.1). The first three axes explained more than 59% of the variation in the 91-dimensional dataset but did not show any clear geographic patterns. When the 427 individuals were grouped based on the geographic area where they were sampled, there was a high degree of overlap among the groups. The two wolf-like individuals with a minority of coyote ancestry stood out as outliers relative to the more coyote-like samples.

The Mantel test of matrix correspondence between linear genetic distance and geographic distance indicated a slight, but significant correlation ($r_M = 0.082$, $P = 0.003$), suggesting a slight signal of isolation by distance. The correlation between genetic distance and log-transformed geographic distance was about the same ($r_M = 0.092$, $P = 0.001$). The test for global spatial autocorrelation revealed that individuals within 50 and 100 km of each other were more genetically similar than expected by chance ($r_G = 0.025$ and 0.003, respectively, $P < 0.05$), but multilocus genotypes of individuals separated by > 100 km were randomly distributed in space (Figure 4.2). Individuals separated by > 350 km were very slightly more genetically dissimilar than expected by chance, again reflecting the weak effect of isolation by distance. The correlogram-wide test of heterogeneity across the distance classes was significant ($\omega = 160.017$, $P = 0.001$), rejecting the null hypothesis of no spatial structure. The local spatial autocorrelation

estimates, r_L, were significantly positive for 27 samples and significantly negative for 3 samples (Figure 4.3). Positive values of r_L were predominantly detected in the same samples, regardless of the number of nearest neighbors in the analysis, thus confirming the consistency of the pattern. However, the localities where there was a significantly positive correlation tended to be those where samples were more clustered geographically, such as in western Pennsylvania, central New York, and northeastern Vermont. For example, six coyotes sampled from the same county in western New Jersey showed a consistent pattern of genetic correlation with their neighbors. On the other hand, two coyotes were negatively correlated with their neighbors even though they were sample from the same locality.

Metapopulation genetic structure

Geneland inferred five or seven genetic populations, depending on the run. Both runs had comparable mean posterior density, but one run inferred two additional populations with zero individuals (i.e., "ghost populations," Guillot *et al.* 2005a). Thus, I chose the run with the simpler, more parsimonious pattern of inferred structure for subsequent analyses. One large population ("Pop 1" in subsequent analyses) was distributed along southern New York, eastern Pennsylvania, New Jersey, and southeastern New England. A second population (Pop2) was distributed along northern New York and northern New England. A third population (Pop 3) was distributed along western New York, western Pennsylvania, and eastern Ohio. A fourth genetic cluster (Pop 4) was geographically disjunct and included a few individuals from western Ohio, and two separate areas of northern New York. Lastly, another spatially disjunct population (Pop 5) consisted of a few coyotes from Cape Cod and Boston, Massachusetts, southwestern Maine, and some related individuals from eastern New York (Figure 4.4a).

Whereas the population assignments suggested patterns of relatedness among individuals (Figure 4.4a), the probabilities of cluster membership revealed patterns of relatedness among populations (Figure 4.4b-f). The groups inferred by Geneland had similar levels of heterozygosity as the total set of 427 canids, but each population had a much higher proportion of loci in Hardy-Weinberg equilibrium (Table 4.2). The increase of loci in Hardy-Weinberg equilibrium indicates the presence of a Wahlund effect in the overall sample and Geneland's ability to describe the underlying population structure. The analysis of molecular variance showed that 99% of the total genetic variation lies within and among individuals, and only 1% of the variation lies among populations. Thus, the extent of genetic differentiation among the five populations is very low, with F_{ST} ranging from 0.006 to 0.039 (Table 4.3). However, despite the low levels of differentiation, every pairwise comparison among the five populations was significant at or below the 0.05 level, which means that no two groups are genetically identical, thus validating the clustering procedure.

Dependence of genetic variation on ecology and geography

The multivariate multiple regression using 365 coyotes (i.e., all samples with complete data on predictor variables) detected highly significant ($P = 0.0001$) relationships between genetic variation and land use, deer density, and geographic distance when considered separately (Table 4.4a). However, each set of variables explained a small proportion of the total genetic variance. All variables together explained only about 5% of the genetic variance. Land use was the set of variables with the greatest explanatory power, yet it only explained 2.1% of the genetic variation. Once the land use variables were fitted in the sequential tests, the next most important predictor was deer density. Regression of individual variables (Table 4.4b) revealed that genetic

distance depended more on longitude (P = 0.0001) than on latitude (P = 0.0444). Among deer density variables, only the highest deer density had a highly significant relationship with genetic distance (P = 0.0001), although the lowest deer density was also marginally significant (P = 0.0497). Among land use variables, only agriculture had a significant effect on genetic differences (P = 0.0158). In the forward-selection modeling procedure, most of the variables added a small, but significant amount to the cumulative proportion of explained genetic variance. Together, longitude and high deer density habitat were the most important individual predictor variables, followed by forested, agricultural, and urban/suburban land use types. Combining the highest two deer density bins did not change the results qualitatively. There was no evidence that the presence of a wolf-derived mitochondrial chromosome influenced nuclear genetic variation at the 91 loci examined.

Discussion

The overall picture that emerges from these results is that coyotes in northeastern North America have a subtle genetic structure that is partially explained by deer density, human land use, and geographic distance. Although coyotes only colonized the region in the last 50-70 years, I found no support for the null hypothesis of no spatial genetic structure, instead finding evidence of genetic subdivisions between populations. This evidence comes from multiple analyses. First, the overall deficiency of heterozygosity relative to expected proportions and the large number of loci out of Hardy-Weinberg equilibrium for the whole sample of 427 individuals (Table 4.2) are expected if there is population structure (i.e., "the Wahlund effect"). The Mantel and spatial autocorrelation tests (Figures 4.2 and 4.3) all pointed to the existence of subtle genetic structure. Geneland was able to further describe the spatial distribution of genetic

variation (Figure 4.4), and although levels of differentiation among the five inferred populations were low, all pairwise comparisons were significant in an AMOVA framework (Table 4.3). Together, these results contradict those of Way *et al.* (2010), who analyzed data from 8 microsatellites genotyped in 340 individuals and concluded that coyotes have a uniform genetic makeup throughout the Northeast. My contradictory results probably reflect the higher resolution achieved with many genetic markers and broader sampling.

The Bayesian analysis of genetic structure, implemented in Geneland, detected the spatial distribution of populations and elucidated a pattern that would have been otherwise impossible to discern. For example, there is no defensible way to bin individuals sampled in this study based on geographical proximity. Similarly, the multivariate ordination of genetic data (Figure 4.1) revealed an almost random distribution of genotypes; so it would not be justifiable to delineate genetic populations based on proximity in principal coordinate space. On the other hand, Bayesian clustering of individuals into populations based on both genetic and geographic data revealed where the main genetic discontinuities lie. Interestingly, the boundaries of four of the five inferred populations lie in central New York, where sampling was the densest (Figure 4.4). New York is the only state where all five populations are represented. One primary genetic discontinuity lies in western New York and Pennsylvania, which is consistent with the recent mixing of two expansion fronts since the 1980s: the northern front that entered New York from Canada over the St. Lawrence River, and the western front that entered the Northeast through Ohio (Fener *et al.* 2005; Kays *et al.* 2010).

The results of the global and local spatial autocorrelation analyses were consistent with the hypothesis of metapopulation structure conforming to a complex mosaic of habitat types. Various patches of positive local autocorrelation were detected in mostly agricultural areas of

Ohio, Pennsylvania, New Jersey, New York, and Vermont (Figure 4.3). These local patches could consist of philopatric individuals related by kinship, or of long distance dispersers with similar habitat affinities. It is possible to have positive and negative correlation at the same locality because several localities had more than one individual, and the local autocorrelation is computed for each individual. The three samples negatively correlated with their neighbors likely represent dispersers from a different population. In the global analysis, only individuals within 100 km of each other were more genetically similar than expected (Figure 4.2); the correlation broke down for pairs of individuals separated by more than 100 km because of the many across-habitat comparisons. Although the correlations were low, they deviated significantly from $r_G = 0$ and were similar in magnitude to other studies evaluating population structure in wild *Canis* (Aspi *et al.* 2006; Stronen *et al.* 2012a).

The metapopulation hypothesis is further corroborated by the more spatially explicit Geneland analysis. One of the inferred groups, Pop 4, is a metapopulation with three disjunct patches of highly agricultural areas in northern Ohio and New York (Figure 4.4). Another group, Pop 5, is made up two disjunct urbanized patches along the coast of New England. The southern patch of Pop 5 is in Cape Cod, which is characterized by urban and suburban development. The northern patch of Pop 5 includes Boston, Massachusetts and Portland, Maine, but extends westward into the Albany, New York area. Both patches of Pop 5 are separated by a forested and agricultural matrix south of the Boston metropolitan area. The westward extension of this metapopulation may be caused a disperser near the Albany area in search of similar urbanized habitat. The distribution of inferred populations depicted in Figure 4.4a is based on modal assignments of pixels within the study area, but in reality there is a probability distribution of any pixel belonging to any of the five inferred groups (Figure 4.4b-f). The areas of high probability

of belonging to one particular group are patchy (e.g., Figure 4.4f), suggesting a complex metapopulation structure in which the habitat of one group is less preferred habitat for another group.

Because all samples were from one decade (1999-2009), I cannot directly estimate if these levels of differentiation among populations are trending upward or downward over time. However, the detailed chronology of range expansion in New York, described by Fener *et al.* (2005), allows me to use space as a proxy for time. A close inspection of pairwise F_{ST} values in Table 4.3 shows that the more "ancestral" and geographically distant populations, Pops 2 and 3 which were established in the 1940s, have a relatively low level of differentiation ($F_{ST} = 0.011$). Most pairwise contrasts among parapatric populations in central New York, which was colonized in the 1960s and 1970s, have higher degrees of divergence. This pattern suggests that some barriers to gene flow may be making recent, adjacent populations of coyotes to be more divergent than the original northern and western colonizers.

What may be the causal mechanisms of such barriers to gene exchange? There is some limited evidence of isolation by geographic distance, but the regression analyses showed that human land use and very high deer density were better predictors of inter-individual genetic distance (Table 4.4). There are not many localities in the study area with very high deer density (exceeding 45 deer per square mile), and only 10 samples represent this density bin. However, combining the two highest density bins does not alter the conclusion: high deer density has a significant relationship with genetic data and is an important predictor that explains genetic variation among individuals. It is interesting to note that admixed northeastern coyotes in areas of high deer density are genetically more wolf-like (Chapter 3). Overall, these results suggest that ecology, more than geography, restricts gene flow in northeastern coyotes. Gene flow in

western coyotes also seems to be restricted by habitat affinities and behavioral, rather than physical, barriers to dispersal (Sacks *et al.* 2004; 2005; 2008). My results show that ecological variation can induce genetic differences when it occurs in a complex, fine-grained mosaic, and not just when it occurs in broad macrohabitat configurations. Part of this ecological variation involves human land use, which has not been considered in previous investigations. Perhaps more importantly, this study shows that ecology-induced genetic differences evolved within the last 30 to 80 years, as long as coyotes have inhabited the Northeast. Previous studies have shown that ecological factors influence population genetic structure in coyotes (Sacks *et al.* 2004; 2005; 2008), wolves (Geffen *et al.* 2004; Pilot *et al.* 2006; Musiani *et al.* 2007; Muñoz-Fuentes *et al.* 2009), *Lynx* (Rueness *et al.* 2003a; 2003b; Reding *et al.* 2012), and hawks (Hull *et al.* 2008), but in all cases, the studied populations have inhabited the study areas for many thousands of years. My results demonstrate the rapid formation of ecological barriers to gene flow in 15 to 40 generations (assuming a generation time of 2 years, Sacks *et al.* 2004). Stronen *et al.* (2012a; 2012b) showed how moderate differentiation can evolve in wolf populations across short distances in about 60 years when the landscape is modified by intense agricultural development. However, an agricultural matrix is highly impermeable to wolf movements, whereas coyotes move readily through natural as well as human-dominated landscapes. The recency of the range expansion into the Northeast and the high permeability of all land use types, including agricultural and urban, may account for the small but significant proportion of genetic variation explained by ecological factors.

The Coyote is a generalist species with great movement potential, thus it is unlikely that there are substantial topographic barriers to dispersal and gene flow in the Northeast. Nonetheless, individuals are not necessarily generalists, but may instead specialize on resources

specific to one habitat type or another (Sacks *et al.* 2004; 2008). Among land use types, all three dominant types explained a significant proportion of genetic variation, suggesting that the relative proportions of forested, agricultural, and developed land influence how individuals cue in on suitable habitat and move, reproduce, and utilize resources within it. However, the perception of suitable habitat is likely to be induced or imprinted by early experiences (Davis & Stamps 2004). For example, natal experience in urban/suburban conditions may habituate a young coyote, so that in its dispersal stage it selects a familiar environment where its individual fitness is maximized. This kind of inter-individual niche variation is common across a broad range of taxa, yet many studies of population dynamics and genetics treat conspecifics as ecologically equivalent (Bolnick *et al.* 2003). High dispersal and gene flow are often thought to prevent local population divergence, but if dispersal is non-random it may actually reinforce evolutionary differentiation (Garant *et al.* 2005). The possibility that overlapping metapopulations occur with their own habitat affinities merits further investigation. This intriguing genetic metapopulation structure may emerge from the strong habitat affinities developed by individuals during their formative period as pups and pre-dispersal juveniles. The habitat-imprinted genetic metapopulation hypothesis may be tested with molecular and telemetry data at a more local scale in a mosaic landscape with the appropriate habitat heterogeneity.

Darwin (1859, p. 90-91) used the relationship of wolves and their prey to illustrate how natural selection may act. Interestingly, his discussion involved a hypothetical scenario of varying deer density and an empirical observation of local adaptation mediated by human land use. Darwin also considered inter-individual niche variation, remarking how, even without any changes in the number of prey, some *individual* wolves have an innate tendency to pursue certain kinds of prey. He noted that two locally adapted varieties of wolf inhabited the Catskill

Mountains of the Northeast; the lighter, swifter type specialized on deer, and the bulky, short-legged type on domestic sheep. The morphological and behavioral adaptations of the sheep specialist must have evolved very rapidly following the expansion of shepherding in the New World during the 17th century. It is interesting that ecological divergence among parapatric populations may occur rapidly, even in long-lived, highly mobile, large predators with low effective population sizes. Apparently, northeastern coyotes may also be rapidly diverging as a response to deer densities and human land use. Whether deer density and land use are mediating a locally adaptive process of ecological and morphological specialization is further explored in Chapter 5.

It is important to note that although I inferred the number and distribution of genetic populations using Geneland, the individual remained the unit of analysis for the regression of multilocus genetic data on predictor variables. This is a departure from most other studies with similar objectives (e.g., Geffen *et al.* 2004; Pilot *et al.* 2006; Musiani *et al.* 2007). There are three main limitations in maintaining the population as the unit of study. One is that often the investigator does not know with certainty the true boundaries of breeding populations, especially in widely distributed and highly ambulatory species. Yet, it remains common practice to bin individuals into predefined groups, mostly based on geographic locality, and perform frequency-based analyses on the basis of the assumed population structure. Bayesian approaches permit the estimation of cluster membership without a priori binning, thus addressing this first limitation. However, while Bayesian methods are well suited to the inference of population structure, they work by maximizing among-group differences, thus neglecting other sources of genetic variation. This is the second limitation: even if the degree of differentiation among populations (whether binned a priori or inferred a posteriori) is significant, much of the genetic (and

ecological) variation undoubtedly remains within populations and within individuals. The third limitation is the tremendous loss of statistical power to detect true associations when the sample size of the analysis is the number of populations rather than the number of individuals. Geffen *et al.* (2004) acknowledged this lack of power to detect effects and interpreted *P*-values < 0.10 as providing evidence against the null hypothesis. For these reasons, taking the approach in which "populations" are not defined for the regression analysis, but instead the individual is the unit of observation, may provide a finer glimpse into population structure and its ecological causes. This approach may work best when the majority of genetic variation remains within populations and individuals, and when population structure is distributed heterogeneously because the underlying ecological variation is distributed in a complex manner. These conditions were certainly met in northeastern coyotes.

Future research should focus on the coyotes of central New York, which is rich with ecological and genetic heterogeneity. To borrow terms from the sociological study of how cultures interact in a heterogeneous society, it would be interesting to investigate whether New York is a "melting pot" or a "salad bowl" of geographical races of eastern coyotes. Noting whether the various populations that share boundaries in central New York retain their distinctiveness over time with growing degrees of differentiation (genetic salad bowl model) or eventually merge into a homogeneous entity (genetic melting pot model) would be an valuable contribution in evolutionary ecology. Increasing differentiation would represent the rapid incipient formation of sympatric and parapatric ecotypes. Also, documenting ecological and evolutionary responses of wild species in an increasingly human-modified world remains an important task.

The results reported here add to the growing number of investigations documenting the induction of cryptic genetic structure by ecological factors. Ecological specialization in parapatric populations of widespread, highly mobile species, even in the absence of physical barriers to dispersal, may be more common than previously appreciated. The local differences in habitat and prey preferences are examples of ecological and behavioral barriers that may promote reproductive isolation and the subsequent formation of varieties, geographical races, subspecies, and closely allied species (Darwin 1866).

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Table 4.1. Predictor variables used to test hypotheses about the influence of geographical distance, ecological variables, and hybridization on patterns of genetic differentiation in northeastern coyotes.

Set	Variables
Geographic distance	Latitude, longitude
Land use	Log-ratio transformed compositional variables representing proportions of forest, agricultural, urban/suburban, and open land cover
Deer density	Categorical variables for four density bins: low, medium, high, very high (< 15, 15-30, 30-45, > 45 deer per square mile)
Hybridization	Indicator variable identifying the presence or absence of a wolf mitochondrial haplotype

Table 4.2. Summary statistics for the full set of 427 canids and five inferred populations by Geneland. N: sample size, H_O and H_E: observed and expected heterozygosity, F: fixation index, HWE: percent of loci in Hardy-Weinberg equilibrium. Values are means over all 91 loci with standard errors in parentheses.

Group	N	H_O	H_E	F	HWE (%)
All	427	0.349 (0.013)	0.379 (0.013)	0.077 (0.013)	70
Pop 1	186	0.359 (0.014)	0.379 (0.014)	0.047 (0.015)	84
Pop 2	85	0.358 (0.014)	0.391 (0.013)	0.081 (0.015)	87
Pop 3	66	0.341 (0.015)	0.365 (0.013)	0.077 (0.022)	84
Pop 4	54	0.311 (0.015)	0.340 (0.015)	0.080 (0.019)	88
Pop 5	8	0.296 (0.021)	0.346 (0.016)	0.109 (0.043)	87

Table 4.3. Pairwise genetic differentiation among five populations inferred by Geneland. F_{ST} values are shown below diagonal and probability values based on 999 AMOVA permutations are shown above diagonal. Despite the low F_{ST} values, all pairwise contrasts were significant at the 5% level, indicating that the Geneland-inferred populations are indeed distinct gene pools.

	Pop 1	**Pop 2**	**Pop 3**	**Pop 4**	**Pop 5**
Pop 1	--	0.005	0.003	0.001	0.034
Pop 2	0.006	--	0.006	0.001	0.043
Pop 3	0.008	0.011	--	0.006	0.038
Pop 4	0.016	0.024	0.008	--	0.009
Pop 5	0.021	0.019	0.023	0.039	--

Table 4.4. Multivariate multiple regression of genetic distances on (a) sets of predictor variables and (b) individual predictor variables to evaluate the effects of geography, ecology, and hybridization on the genetic structure of northeastern coyotes. Marginal tests consider each variable or variable set individually, ignoring all others. Sequential tests include in the model one variable or variable set at a time, conditioned on variables already in the model. The top-down sequence of variables or variable sets corresponds to the sequence indicated by the forward-selection procedure. *P*-values less than 0.05 are highlighted in bold. %Var: percentage of the multivariate genetic variance explained by variable or variable set, Cum%Var: cumulative percentage of variance explained in forward-selection procedure.

	Marginal tests			Sequential tests			
Variable or Variable Set	***F***	***P***	**%Var**	***F***	***P***	**%Var**	**Cum%Var**
(a) Regression on sets of variables fitted singly and sequentially							
Land use	1.932	**0.0001**	2.1%	1.932	**0.0001**	2.1%	2.1%
Deer density	0.952	**0.0001**	1.1%	1.696	**0.0011**	1.8%	3.9%
Geographic distance	2.462	**0.0001**	1.3%	0.752	0.13	0.4%	4.3%
Hybridization	0.869	0.6704	0.2%	0.958	0.5171	0.3%	4.6%
(b) Regression on individual variables fitted singly and sequentially							
Longitude	2.921	**0.0001**	0.8%	2.921	**0.0001**	0.8%	0.8%
Deer density very high	2.875	**0.0001**	0.8%	2.781	**0.0003**	0.8%	1.6%
Forest	1.171	0.2541	0.3%	1.790	**0.0075**	0.5%	2.0%
Agricultural	1.675	**0.0158**	0.5%	1.627	**0.0211**	0.4%	2.5%
Urban/suburban	1.213	0.2103	0.3%	1.930	**0.0019**	0.5%	3.0%
Latitude	1.509	**0.0444**	0.4%	1.501	**0.0466**	0.4%	3.4%
Open	1.364	0.1028	0.4%	1.375	0.0951	0.4%	3.8%
Deer density high	1.403	0.0808	0.4%	1.243	0.1841	0.3%	4.1%
Deer density medium	1.192	0.2209	0.3%	1.042	0.4113	0.3%	4.4%
Wolf mtDNA	0.869	0.6704	0.2%	0.914	0.5935	0.3%	4.6%
Deer density low	1.482	**0.0497**	0.4%	0.000	1	0.0%	4.6%

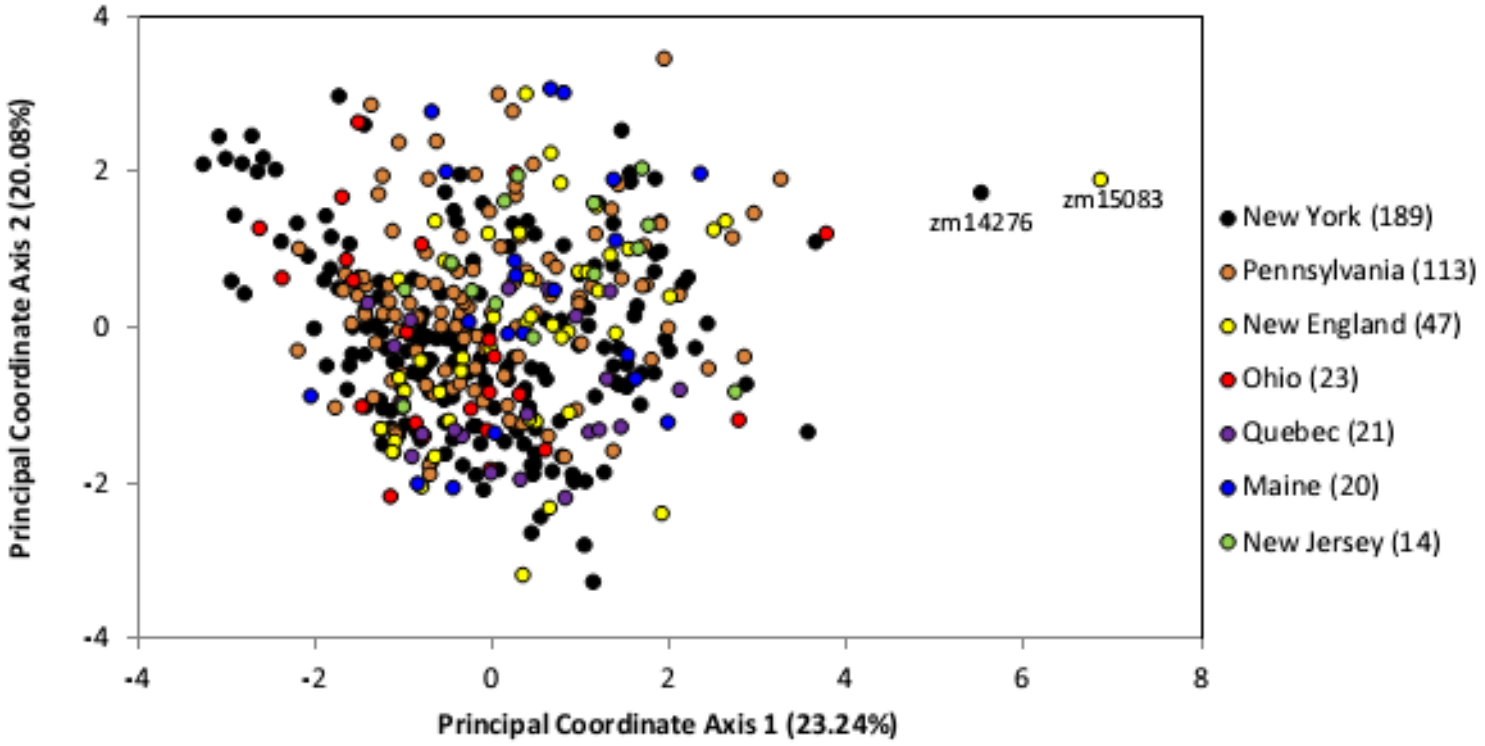

Figure 4.1. Biplot of principal coordinates analysis of genetic variation in northeastern canids. The first two axes explain 43.33% of the total variation. All 427 samples are plotted by state or province, with sample size in parentheses. The New England group includes Connecticut, Rhode Island, Massachusetts, Vermont, and New Hampshire. The two outliers in axis 1 are wolf-like individuals from Vermont and New York.

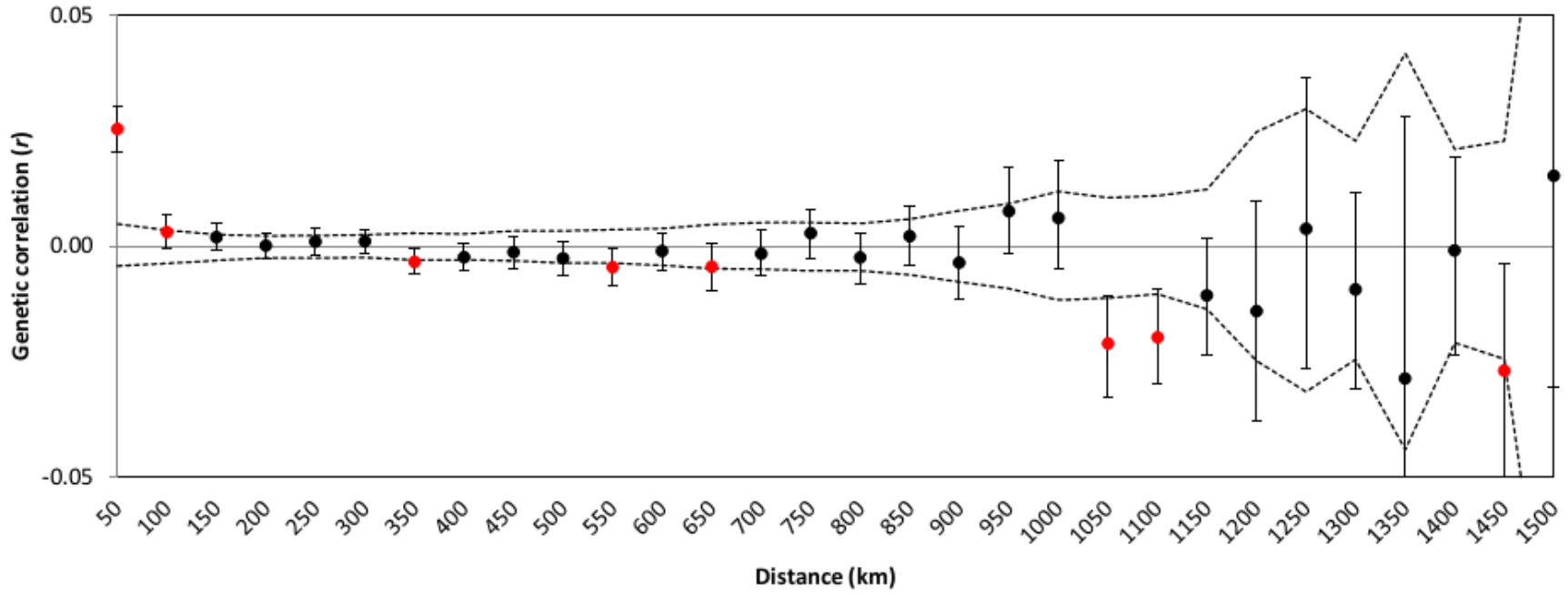

Figure 4.2. Correlogram plotting the global autocorrelation coefficient (r_G) as a function of geographic distance. The error bars indicate the bootstrapped 95% confidence interval of *r*, and the dashed lines indicate the permuted 95% confidence interval of the null hypothesis. Red data points indicate significant positive or negative deviations from r = 0. The correlogram-wide test of heterogeneity was significant ($\omega = 160.017$, $P = 0.001$).

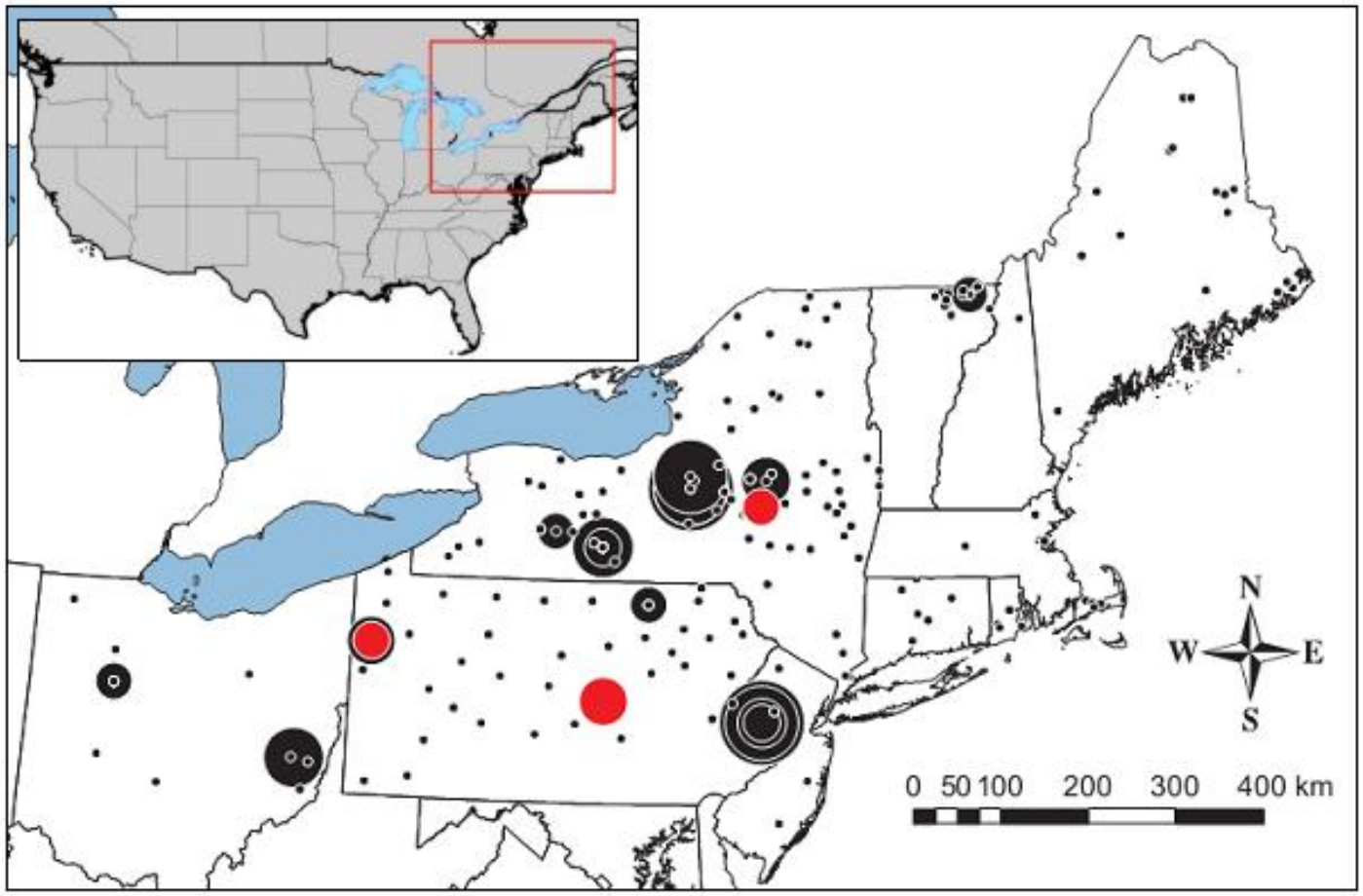

Figure 4.3. Bubble plot of local spatial autocorrelation analysis with 399 individuals plotted as points. Larger bubbles indicate localities with significant positive (black) and negative (red) local autocorrelation. Bubble size is proportional to the number of runs in which r_L was significant ($P < 0.001$), thus indicating consistency of correlation signal when r_L was computed by sampling 4, 8, 12, 16, and 20 nearest neighbors. Individuals positively correlated with their neighbors are likely philopatric, whereas those negatively correlated with their neighbors are likely dispersers from a different population.

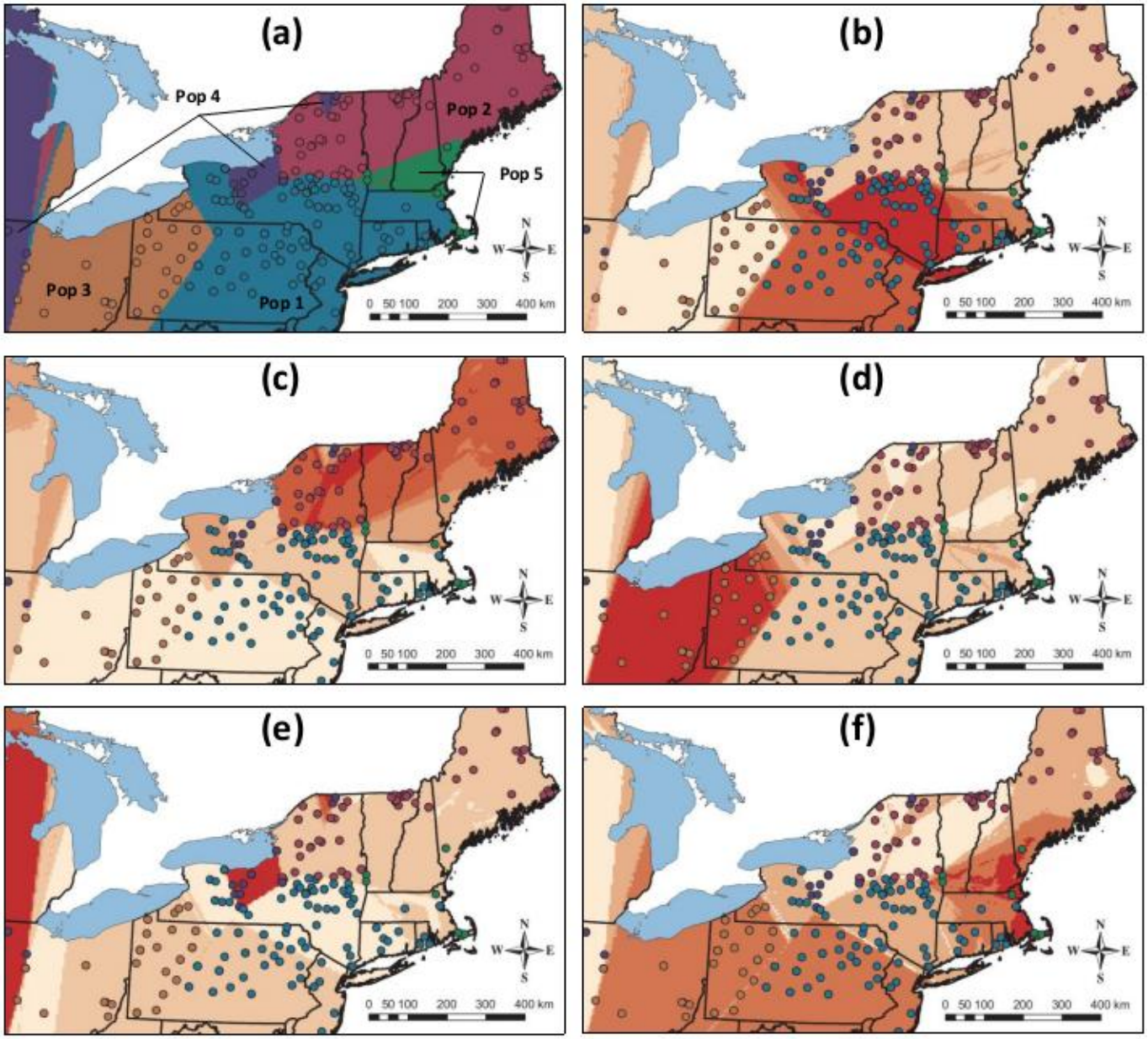

Figure 4.4. Bayesian clustering of individuals into populations based on genetic and geographic data. (a) Spatial distribution of five inferred populations based on modal assignment of individuals and pixels in the study area. Pop 4 and 5 are metapopulations consisting of three and two disjunct patches, respectively. (b-f) Posterior probability of belonging to clusters 1 to 5, respectively, for all pixels in the study domain. Darker shades of red indicate higher probabilities.

CHAPTER 5

LOCAL ADAPTATION OF NORTHEASTERN COYOTE ECOTYPES MEDIATED BY DEER: AN ANALYSIS OF 5 FUNCTIONAL SNPS

"Wolves inhabiting a mountainous district, and those frequenting the lowlands, would naturally be forced to hunt different prey; and from the continued preservation of the individuals best fitted for the two sites, two varieties might slowly be formed…. [T]here are two varieties of the wolf inhabiting the Catskill Mountains in the United States, one with a light greyhound-like form, which pursues deer, and the other more bulky, with shorter legs, which more frequently attacks the shepherd's flocks."

- Charles Darwin, *On the Origin of Species* 1859

Abstract

More than 150 years after Darwin's theory of natural selection, it remains difficult to determine whether populations diverge due to neutral or adaptive processes. Adaptation requires that natural selection act on ecologically and functionally important genes, so one way to disentangle these evolutionary forces is to compare genetic differences between populations at functional loci. By genotyping five single nucleotide polymorphisms (SNPs) that are associated with ecologically important morphological traits, I examined the molecular basis of local adaptation in a broad geographic sample of 427 northeastern canids that represent a hybrid swarm with coyote (*Canis latrans*), wolf (*C. lupus*), and dog (*C. familiaris*) ancestors. I provide the first documented evidence of any wild canids with homozygous mutant genotypes in these five SNPs

which were ascertained in domestic dogs. Multivariate analyses of skull measurements show that coyotes with mutant genotypes are morphological outliers or peripheral individuals, indicating a clear association of morphological and genetic variation. Analyses of molecular variance reveal that strong diversifying selection, mediated by deer densities, is acting on two loci related to body size and skull morphology, and that differentiation is occurring in habitats with high densities of deer. These results suggest that a localized area of high deer density is mediating morphological adaptation and ecological specialization in coyotes.

Introduction

How populations diverge is of great interest because it represents the incipient stage of speciation, whether by adaptation to different local environments (Darwin 1859) or by neutral evolutionary processes (Lynch & Hill 1986). With the advent of molecular tools, it has become relatively simple to estimate levels of divergence among populations (Whitlock & McCauley 1999); yet it remains difficult to distinguish between the mechanisms of ecological adaptation and neutral evolution (Eizaguirre *et al.* 2011) despite the longstanding impetus to do so (Lewontin & Hubby 1966; Lewontin & Krakauer 1973). This endeavor is especially challenging in species with high levels of dispersal because gene flow can be both a constraining and a creative force in adaptive evolution (Slatkin 1987; Garant *et al.* 2005). Because natural selection can establish local differences much more effectively and rapidly than genetic drift, one way to disentangle these evolutionary forces is to compare genetic differences between populations at a few loci putatively under selection versus other neutral or nearly neutral loci (Slatkin 1987).

Different habitat types impose different selective pressures on organisms (Davis & Stamps 2004). This spatial variation in selection acts as a partial barrier to gene flow (Slatkin

1987), so that differential local selection is expected to drive the evolution of *ecotypes* – genetically or phenotypically distinct populations that are adapted to their local environment or particular type of habitat (Futuyma 1998, p. 254). But if the spatial scale of dispersal exceeds the scale of environmental heterogeneity, then gene flow is expected to limit local adaptations. It then remains puzzling how widely distributed and highly vagile species diverge.

Evidence is mounting that large canids form parapatric ecotypes despite their high mobility and potential for gene flow. In California, adjacent populations of coyotes (*Canis latrans*) are genetically differentiated despite the absence of any known dispersal barriers, and the genetic subdivisions correspond to California's bioregions (Sacks *et al.* 2004). Apparently, natal experience induces habitat preferences in individuals so that across-bioregion movement is limited because coyotes seek similar habitat as they disperse. Habitat specialization appears to be so strong that coyotes in the Mojave Desert are more related to coyotes in the arid short-grass prairie of Colorado (more than 1000 km distant) than to adjacent populations in other habitat types of California (Sacks *et al.* 2008). Northeastern coyotes also form parapatric metapopulations, and some of the genetic variation is explained by human land use and density of one of their primary prey, white-tailed deer (*Odocoileus virginianus*) (Chapter 4). Similarly, two varieties of wolf formerly inhabited the Catskill Mountains in the United States; their ecological and morphological divergence was mediated by specializations related to deer and human land use (Darwin 1859). Genetic differentiation among European grey wolves (*Canis lupus*) is not explained by topographic barriers nor past fragmentation, but by habitat types, climate, and diet (Pilot *et al.* 2006). Likewise, in Canada, boreal forest wolves are genetically and phenotypically distinct from the neighboring tundra wolves (Musiani *et al.* 2007); and at a much finer spatial scale, the coastal wolves of British Columbia are morphologically,

behaviorally, and genetically distinct from nearby inland wolves (Muñoz-Fuentes *et al.* 2009). In all these examples, genetic subdivisions were associated with ecological variation and/or local foraging specializations.

Adaptation requires that natural selection act on ecologically and functionally important genes. Although the aforementioned genetic patterns described in coyote and wolf are suggestive of an adaptive process, they were detected using neutral microsatellite, mitochondrial DNA (mtDNA), and single nucleotide polymorphism (SNP) markers. Traditionally, the application of genetics to wildlife biology and conservation rests on the premise that neutral marker variation in populations reflects levels of adaptive genetic variation (Kohn *et al.* 2006). Studies interrogating genetic markers that are selectively important (i.e., non-neutral) or linked to other loci under selection can lead us to better understand the genetic basis of local adaptation, but such studies are scarce. One way to address the molecular basis of local adaptation is to select genetic markers from a known association with functional genes. Inclusion of such markers ensures that they are found within or linked to candidate genes that are probable targets of selection (Kohn *et al.* 2006); this approach to identifying gene regions under selection is more practical than whole-genome scans (Morin *et al.* 2004). The availability of the dog (*Canis familiaris*) genome sequence makes the coyote, the sister species of the dog, a "genome enabled taxon" (Kohn *et al.* 2006). This means that genetic or genomic tools developed for the dog, a model organism, may be utilized to explore questions in closely related wild canids (Ostrander & Wayne 2005; Wayne & Ostrander 2007).

The growing field of canine genetics is shedding light on genotype-phenotype associations of behavioral and morphological traits, some of which may be relevant to fitness in the wild. For example, various quantitative trait loci (QTLs) associated with body size and skull

morphology have been identified (Haworth *et al.* 2001; Sutter *et al.* 2007; Jones *et al.* 2008). Body size and skull morphology are two important traits that influence individual fitness in vertebrate predators. Body mass appears to be a strong determinant in feeding ecology, with carnivores < 20 kg feeding primarily on small prey, and carnivores > 20 kg specializing on large vertebrate prey (Carbone *et al.* 1999; Carbone *et al.* 2007). Northeastern coyotes are at the margin of this body mass threshold, and selection for body mass may vary depending on the prey species associated with different habitats. Skull morphology is also largely associated with diet. Although the coyote is described as a dietary generalist (Bekoff 1977), the term *generalist* more accurately describes the species. Locally adapted ecotypes are more likely to be dietary specialists, comprised of individuals with preferences shaped by natal experience, natural selection, or both (Bolnick *et al.* 2003). For example, coyote-wolf hybrids in northeastern forests have more robust skulls than western coyotes (Kays *et al.* 2010) and other eastern coyotes in more disturbed habitats (Sears *et al.* 2003). These morphological differences in skull dimensions are thought to be adaptations that confer advantages for capturing and killing deer (Kays *et al.* 2010).

The objective of this study was to investigate the molecular basis of local adaptation in an ecologically heterogeneous landscape by using genetic markers putatively under selection. Here I present data on five functional SNPs genotyped in a broad geographic sample of 427 wild northeastern coyote-wolf-dog hybrids. The five SNPs are mutations discovered in domestic dogs and are simple Mendelian loci that are strongly associated with body size and skull size. I also compare the morphological patterns of variation in a subset of 47 northeastern canids in relation to their genotypes at the five SNPs. With these data I test the hypothesis that northeastern canids occur as ecotypes adapted to local prey or human land use conditions. Specifically, I predict that

(1) wild canids with mutant genotypes in the five functional SNPs are morphological outliers or peripheral individuals, (2) frequencies of mutant alleles associated with smaller body and skull size are dependent on density of large prey or land use, and (3) genetic differentiation in the five functional genes will differ significantly from differentiation in other loci representative of the whole genome. The five genes considered in this study are ecologically and functionally important in the wild (e.g., for prey specializations and hunting success) and may influence individual fitness. Although these functional genes have been studied in the domestic dog, this is the first spatially-explicit, population-level interrogation of these genes in a wild, non-model organism.

Methods

Study area and sampling

The study area and specimens I used in this analysis of local adaptation were described in Chapter 3. Ohio and the Northeast are characterized by a mosaic of land cover and land use types, with forested land, agricultural land, and urban/suburban developed land being the most dominant and most different types. Two of the 427 specimens were more wolf-like genetically and morphologically, but all others were, on average, of 63% coyote ancestry (Chapter 3), so I refer to these hereafter as coyotes. Coyotes and white-tailed deer are continuously distributed throughout the region.

Selection of functional SNPs

I genotyped five SNPs associated with functional morphological traits. Three SNPs are QTLs associated with body size in *Canis*: *IGF1*, *SMAD2*, and *IGF2BP2*; two SNPs are QTLs

associated with skull morphology: *IGFBP4* and *TCOF1* (Table 5.1). *IGF1* is a 72-kbp gene that codes for the insulin-like growth factor 1 protein. *IGF1* has many polymorphic sites, but I genotyped a SNP proximate to the causative mutation for small body size in dogs (Sutter *et al.* 2007). *SMAD2* and *IGF2BP2* are candidate genes thought to be involved in the regulation of body size (Jones *et al.* 2008). I genotyped one SNP 680 bp upstream of *SMAD2* and one SNP in an intron of *IGF2BP2*. *IGFBP4* is a quantitative trait locus associated with head and body proportions (Jones *et al.* 2008). The SNP I genotyped is 76 kbp from and in linkage disequilibrium with *IGFBP4*. *TCOF1* encodes treacle protein, which functions in craniofacial development. I genotyped the SNP that represents a missense (non-synonymous) mutation in exon 4 and is associated with brachycephaly (broad skull and short face) in dogs (Haworth *et al.* 2001). The five functional SNPs were genotyped with the same Illumina GoldenGate multiplex assay described in Chapter 3. The laboratory methods and processing of raw data for quality control are also described in Chapter 3.

Association of genetic and morphological variation

I used R (R Development Core Team 2012) to perform a principal components analysis (PCA) of 10 skull traits measured in 47 individuals that were also in my genetic sample. The morphological traits and their measurements were described in Kays *et al*. (2010). I used the traditional PCA biplot of the first two eigenvectors to visualize the distribution of morphological variation by sex. I also used boxplots to visualize the distribution of phenotypes for each of three genotypes per SNP.

Association of genetic and ecological variation

I used PLINK 1.07 (Purcell *et al.* 2007; http://pngu.mgh.harvard.edu/purcell/plink) to calculate allele frequencies in different classes of habitat based on either density of white-tailed deer or human land use. I obtained deer density data from Quality Deer Management Association (http://www.qdma.com/shop/qdma-white-tailed-deer-density-map). The QDMA map depicts deer densities across management units or counties and summarizes data provided by state wildlife agencies. I obtained land use data from the US Geological Survey Land Cover 2001 Database (http://www.mrlc.gov/nlcd2001.php) and extracted the proportions of the various land use classes for each sample in two different ways, depending on the precision of a sample's georeferenced coordinates, as described in Chapter 4. For the deer density analyses, individuals were assigned to the density class on which their georeferenced coordinate landed. However, because the resolution of the land use data is so high (30-m × 30-m), for the land use analyses, individuals were assigned to a dominant land use class in two ways: (1) if any land use class represented more than 50% of the area around the georeferenced coordinate, the individual was assigned to that land use class; (2) if urban/suburban land represented more than 20% of the area around the georeferenced coordinate, the individual was designated an urban/suburban coyote, and all remaining individuals were designated as forest coyotes or agricultural coyotes if that land use class represented more than 50% of the area around the georeferenced coordinate. In both methods of assignment, individuals who did not meet those a priori thresholds were excluded from analyses relating genetic and ecological variation.

I performed G-tests of independence in GenAlEx 6.5 (Peakall & Smouse 2006; 2012) to examine whether the frequencies of mutant alleles are dependent of deer density and land use. To test for genetic differences among "populations" delineated a priori by deer density or land

use, I calculated pairwise genetic distance between alleles for each of the five functional loci and then performed an analysis of molecular variance (AMOVA) using GenAlEx 6.5. AMOVA is a distance-based analysis that allows the hierarchical partitioning of genetic variation among populations, among individuals within populations, and within individuals. The analysis calculates F_{ST}, a global measure of genetic divergence, as the proportion of the genetic variance among populations relative to the total variance. I assessed the significance of the estimated F_{ST} values using 999 random permutations. For direct comparison, I performed a separate AMOVA for the 91 SNPs in Chapter 4 that represent the whole genome. If the locus-specific global F_{ST} was significantly greater than zero at the 0.01 level, I calculated pairwise F_{ST} and assessed the significance of the pairwise population differences also using 999 random permutations.

Results

Genotyping results and novel discovery of wild homozygous mutants

I genotyped five functional SNPs in 425 northeastern coyotes and 2 immigrant wolves. Total genotyping rate was 97.2%. The mutant alleles are rare with frequencies below 0.05 for three of the five SNPs (Table 5.2). Homozygous mutants are also very rare, but because of the large sample size, thirteen unique individuals were homozygous mutants in at least one of the five genes. These homozygous mutants were sampled from New York, New Jersey, Pennsylvania, Maine, and Massachusetts. One individual, zt226, was a double homozygote for the *SMAD2* and *IGF2BP2* mutations. One large individual, zm14276, was an immigrant wolf found in Saratoga County, New York (Chapter 3), and was homozygous for the rare G allele of *SMAD2* associated with small size. Although the frequency of the mutant allele of *TCOF1* was higher than that of the other four SNPs, no homozygous mutants were present in our large

sample. This is the first documented evidence of wild canids with homozygous mutant genotypes in these five SNPs which were ascertained in domestic dogs.

Association of genetic and morphological variation

In an initial PCA of skull measurements from all 47 individuals represented in both genetic and morphologic datasets, the first two principal components explained 74% of the variation, collectively. Two individuals were extreme outliers: zm14276, a suspected male wolf who was homozygous for the *SMAD2* mutation, and zm15173, a female who was 32% wolf (Chapter 3). In a subsequent PCA excluding the two outliers, the first and second principal components explained 44% and 14% of the morphological variation, respectively (Figure 5.1a). The first component was clearly a size axis and male coyotes were generally larger than females, with modest overlap. The two remaining male homozygous mutants were on the periphery of the male cluster and toward the lower end of the distribution; the sole female homozygous mutant was also on the periphery of the female cluster, but on the higher side of the distribution.

When considering each genotype individually, each of the four homozygous mutants was a morphological outlier or peripheral individual among its peers of the same sex (Figure 5.1b). Whereas the *IGF1* and *IGFBP4* mutants were on the predicted side of the distribution (i.e., smaller size), the *SMAD2* and *IGF2BP2* mutants were on the "wrong" side of the distribution (i.e., larger size). For *SMAD2* and *IGF2BP2*, the heterozygous genotype appeared to have no phenotypic difference from the wildtype genotype, but the *IGF1* heterozygote displayed a phenotype more similar to that of the mutant homozygote.

Association of genetic and ecological variation

Allele frequencies of *SMAD2*, *IGF2BP2*, and *TCOF1* were not independent of deer density (G-test of independence *P*-values: *SMAD2* = 0.001, *IGF2BP2* = 0.035, *TCOF1* = 0.042) (Figure 5.2a). The mutant alleles of *SMAD2* and *TCOF1* were significantly overrepresented in areas of very high deer density. Allele frequencies of the other two genes, *IGF1* and *IGFBP4*, were independent of deer density (*P*-values: *IGF1* = 0.107, *IGFBP4* = 0.377). The tests of independence were marginally significant for *SMAD2* (*P* = 0.051), *IGF2BP2* (*P* = 0.087), and TCOF1 (*P* = 0.070) when the two highest deer density bins were pooled. Allele frequencies of all five SNPs were independent of land use, regardless of the method of assigning individuals to land use classes (*P*-values for method 1: *IGF1* = 0.082, *SMAD2* = 0.163, *IGF2BP2* = 0.673, *IGFBP4* = 0.874, *TCOF1* = 0.377.).

Genetic differentiation among populations delineated by deer density class was significant for *SMAD2* and *TCOF1* (Table 5.3). The F_{ST} estimates for these two genetic markers (*SMAD2*: 0.327, *TCOF1*: 0.113) were highly significant and much greater than the estimates for the other 3 functional loci (*IGF1*: 0.003, *IGF2BP2*: 0.052, *IGFBP4*: 0) and for the 91 SNPs representative of the whole genome (average: 0.004, range: 0-0.036). Upon further inspection of pairwise differences, it became evident that coyotes in the highest deer density habitats are highly differentiated from coyotes in lower deer density habitats in respect to *SMAD2* and *TCOF1*, with F_{ST} estimates as high as 0.5 (Table 5.4). The global F_{ST} estimates for *SMAD2* and *TCOF1* were not significant when the two highest deer density bins were pooled, but pairwise F_{ST} comparisons consistently showed significant differentiation of coyotes in high deer density habitats. There was no significant genetic differentiation between populations delineated by land

use regardless of assignment method, neither at the background genome level nor at any of the functional loci (Table 5.3).

Discussion

The results of this study support the hypothesis that northeastern coyotes occur as locally adapted ecotypes and begins to describe the causal mechanism for specialization. The evolution of ecotypes necessitates some degree of ecology-mediated reduction of gene flow and functional variation on which selection can act. In this case, the reduction of gene flow is mediated by the density of white-tailed deer, a main prey species, or another correlated environmental factor; and the target of selection appears to be the genetic architecture that underlies morphological traits related to predation.

Several lines of evidence support the role of natural selection acting on morphogenetic variation. First, there was a clear association of morphological and genetic variation at the functional loci examined. All four canids with mutant functional SNP genotypes for which we had morphological samples were outliers or peripheral individuals. The mutant genotype of *SMAD2* is correlated with small body size in dogs, so it was initially surprising to see the only *SMAD2* mutant as an extreme outlier toward the "large" end of the distribution. However, the outlier phenotype of the *SMAD2* mutant was more likely due to its 70% wolf ancestry (Chapter 3) then to its *SMAD2* genotype. It would be interesting to genotype many wolves at the *SMAD2* locus to test if the GG genotype is indeed associated with small body size.

Second, a statistical test of independence revealed that frequencies of mutant alleles associated with smaller body and skull size are dependent on deer density. Coyotes living in areas with high densities of deer are more likely to possess the mutant alleles associated with

smaller body and skull size. The non-independence was expected, but not the direction. It was surprising to observe relatively high frequencies of the mutant alleles of *SMAD2* and *TCOF1* in areas of high deer density. The high incidence of the mutant alleles – and presumably a smaller or brachycephalic phenotype – in deer dense habitats may be explained by at least three plausible scenarios. High densities of deer may actually make it easier for coyotes to hunt them, especially their fawns, thus relaxing selection on body and skull size. Northeastern coyotes do exhibit a positive functional response to increasing deer density, and predation of fawns is much greater in areas of high deer density versus areas of low deer density (Blanton & Hill 1989; Patterson *et al.* 1998). Theory predicts that a higher density of prey would make it easier to hunt only initially until the predator-prey ratio equilibrates, and then it would get just as difficult to hunt deer. However, extrinsic factors, such as intense harvesting, may prevent this stabilization. Alternatively, highly productive habitats that support many deer per unit area may also support a high density of other prey species, so that coyotes living in them have a more generalized diet, thus relaxing selection favoring deer-hunting specializations. This hypothesis could be tested by analyzing the stomach contents or scat of coyotes across habitats to verify that deer consumption is related to abundance of other prey or inversely proportional to deer density. While the hypothesis seems plausible, it is not supported by some studies. Occurrence of deer in coyote scats is not correlated with rabbit and rodent abundance (Grogan 1996) and is greater in areas of high deer density versus areas of low deer density (Blanton & Hill 1989). Or perhaps, deer are reaching high densities in these areas because the smaller coyotes there are not apt to killing them. Some studies showed that coyotes are more effective deer hunters in areas of low deer density, probably because deer reduce their vulnerability to coyote predation by congregating in high densities (Messier & Barrette 1985; Patterson & Messier 2001). Many factors influence the

killing rates of deer by coyotes, such as winter severity and coyote social behavior (Patterson & Messier 2000), but it seems that coyotes are more capable predators of fawns when deer are dense, and more capable predators of adults when deer are sparse. The scenarios proposed to explain the disproportionately high frequency of the *SMAD2* and *TCOF1* mutations in deer dense habitats are speculative at this point and would need to be supported by further research.

Third, analyses of molecular variance corroborated the aforementioned tests of independence, showing that genetic differentiation is not significant with respect to human land use, but is significant with respect to deer density, specifically at the *SMAD2* and *TCOF1* loci. Contrasted to background genome-wide levels, significantly higher F_{ST} is indicative of diversifying selection, whereas significantly lower F_{ST} is indicative of homogenizing or balancing selection (Lewontin & Krakauer 1973; Beaumont 2005). The very high estimates of F_{ST} for *SMAD2* and *TCOF1* indicate that strong diversifying selection is acting on these or closely linked loci, favoring different alleles in different habitats (Slatkin 1987). Pairwise contrasts among deer density classes further revealed that the differentiation is occurring exclusively in habitats with the highest density of deer. It is worth noting that separate analyses with a separate set of SNPs demonstrated that coyotes living in areas of high deer density appear more wolf-like genetically (Chapter 3), and that high deer density is an important predictor of genetic variation (Chapter 4), supporting the idea that hybridization with wolves introduced adaptive genetic variation (Kays *et al.* 2010).

These lines of evidence converge to suggest that a localized area of high deer density is mediating morphological adaptation and ecological specialization in northeastern coyotes. One caveat is worth mentioning. The sample size and geographic extent of the highest deer density class were rather small, consisting of ten coyotes sampled from three localities in New Jersey.

This was primarily a constraint of the regional variation of deer abundance and the way density data were classified by QDMA. There are large contiguous areas where deer occur at densities exceeding 45 deer/mile^2 in Michigan, Wisconsin, Texas, and Mississippi; but such extreme densities are rare in the Northeast, occurring patchily in New Jersey, Massachusetts, and Ohio. It is possible that the ten sampled coyotes were closely related, but this does not oppose the conclusion of a locally adapted ecotype. Immigrant inviability, assortative mating, or dispersal affinities biased towards the natal habitat – mechanisms of natural selection that reduce gene flow across phenotypes and habitats – are expected to make individuals closely related within a habitat type (Davis & Stamps 2004; Sacks *et al.* 2004; Nosil *et al.* 2005). A neutral or purely demographic process does not explain the very high F_{ST} estimates for *SMAD2* and *TCOF1* relative to the very low estimates across the rest of the genome. Moreover, the AMOVA framework of hypothesis testing via many random permutations removes the uncertainty of the observed variance possibly being due to chance. If deer density truly is driving genetic divergence, one might expect a clinal pattern of F_{ST} across density classes and a consistency of results when the two highest deer density bins are combined. The data demonstrate this pattern: coyotes in the highest deer density habitats are highly differentiated from those in the lower density habitats, but less differentiated from those in the second highest density habitat (Table 5.4). And the results for *SMAD2*, *TCOF1*, and *IGF2BP2* closely approached significance when the two highest deer density classes were combined. Although the deer density variable considered here was categorical, there is an underlying continuous distribution. Sampling across a more fine-grained gradient of ungulate density may uncover more precisely the influence of prey densities on genetic variance.

Natural selection requires heritable phenotypic variation on which to act. Here I showed evidence for the footprint of selection on at least two functional variants related to ecologically important morphological traits. Future studies should focus on the origin of this variation in coyotes. It has been posited that hybridization with wolves introduced adaptive genetic variation into the genome of coyotes (Chapter 3; Kays *et al.* 2010), but the introgression of adaptive variation via hybridization with dogs is a possibility that merits more research. Anderson *et al.* (2009) showed that a mutation in the *K* locus of the melanocortin pathway, which causes melanism in the gray wolf, originated in dogs and is favored in forested habitats. Finding other mutations associated with morphological characters in wild canids has been difficult. The mutant alleles discovered in genome-wide association studies of dog breeds are mostly absent in wolves and coyotes (e.g., Boyko *et al.* 2010). The present study is the first to describe the existence of wild canids with homozygous mutant genotypes in the five SNPs that I examined. These SNPs were discovered in domestic dogs, and previous to this study, only dogs were thought to carry two copies of these mutations. Admixture with dogs is widespread in the Northeast (Chapter 3), so it is likely that coyotes acquired these mutations from dogs.

The results of this study validated the implicit assumption that a simple genetic architecture underlies morphological variation in wild canids, as in domestic dogs, thus opening an opportunity for future molecular investigations of morphological adaptation in wild *Canis*. I presupposed that SNPs associated with morphological variation in domestic dogs are also associated with morphological variation in wild dogs. In contrast to the complex genetic architecture underlying quantitative traits in humans and domestic plants, Boyko *et al.* (2010) showed that a small number of quantitative trait loci of large effect explain much phenotypic variation in dogs. My results are consistent with this simple model of dominant-recessive QTLs

of large effect on phenotypic variation. A potentially fruitful avenue of research would be to investigate the molecular basis of adaptation in other traits with a simple mode of inheritance. For example, coat color in domestic and wild canids is largely determined by variation in a small number of genes: the agouti (*A* or *ASIP*), brown (*B* or *TYRP1*), extension (*E* or *MC1R*), and black (*K* or *CBD103*) loci (Schmutz & Berryere 2007; Schmutz *et al.* 2007). Coat color diversity appears to be under differential selection in wild canids, and may be related to hunting specializations in various habitats (Musiani *et al.* 2007; Anderson *et al.* 2009). Also, musculature and running speed in dogs are largely affected by a 2-bp indel mutation in myostatin (*MSTN*), a gene involved in muscle growth (Mosher *et al.* 2007). This gene has not been studied except in model organisms such as mice, cattle, dogs, and humans. Although one study examined *MSTN* in a few wild canids (Grzes *et al.* 2009), it was primarily a comparative cytogenetics analysis. It would be interesting to compare *MSTN* variants across various habitats to test whether wolf-like canids tend to be faster and more muscular where there are more large-bodied prey. Lastly, it would be useful for conservation geneticists to uncover the genetic basis of maladaptation or of morphological deformities, such as those exhibited by the inbred wolves of Isle Royale, Michigan (Räikkönen *et al.* 2009).

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Table 5.1. Five single-nucleotide polymorphisms in or linked to functional genes associated with ecologically important morphological traits.

Gene	SNP	Protein	Trait	Description	Reference
IGF1	rs22437444	Insulin-like growth factor 1	Body size	SNP is proximate to causative mutation that diminishes body size in dogs	Sutter *et al.* 2007
SMAD2	rs24445718	SMAD family member 2	Body size	SNP is near *SMAD2,* a QTL associated with height and weight in dogs	Jones *et al.* 2008
IGF2BP2	rs23872573	Insulin-like growth factor 2 binding protein 2	Body size	SNP is in an intron of *IGF2BP2,* a QTL associated with height and weight in dogs	Jones *et al.* 2008
IGFBP4	rs24564560	Insulin-like growth factor binding protein 4	Skull morphology	SNP is near *IGFBP4,* a QTL associated with head-to-body ratio in dogs	Jones *et al.* 2008
TCOF1	rs24098843	Treacher Collins syndrome protein, treacle protein	Skull morphology	Non-synonymous mutation in coding region of gene expressed in craniofacial bones	Haworth *et al.* 2001

Table 5.2. Mutant genotype counts and frequencies of five functional SNPs. MAF: mutant allele frequency, AA: homozygous mutant genotype, AB: heterozygous genotype, BB: homozygous wildtype genotype.

Gene	SNP	Homozygous Mutant Count	MAF	AA	AB	BB
IGF1	rs22437444	2	0.036	0.005	0.063	0.933
SMAD2	rs24445718	5	0.048	0.012	0.072	0.916
IGF2BP2	rs23872573	6	0.050	0.014	0.072	0.914
IGFBP4	rs24564560	1	0.046	0.002	0.087	0.911
TCOF1	rs24098843	0	0.084	0	0.168	0.832

Table 5.3. Genetic differentiation of coyote populations when subdivided a priori by deer density or human land use. Global F_{ST} and *P*-values are given for 5 functional genes and 91 SNPs representative of the whole genome; bold type: $P \leq 0.01$.

	Deer density		**Land use**	
Genetic marker(s)	**F_{ST}**	***P***	**F_{ST}**	***P***
IGF1	0.003	0.332	0.042	0.038
SMAD2	**0.327**	**0.001**	0.005	0.313
IGF2BP2	0.052	0.054	0	0.776
IGFBP4	0	0.482	0	0.909
TCOF1	**0.113**	**0.007**	0	0.368
91 variable SNPs	0.004	0.149	0.002	0.295

Table 5.4. Locus-specific pairwise genetic differentiation of coyotes in different habitats classified by deer density. F_{ST} values are shown below diagonal and probability values based on 999 AMOVA permutations are shown above diagonal; bold type: $P \leq 0.005$.

	SMAD2				***TCOF1***			
Deer/mile^2	**<15**	**15-30**	**30-45**	**>45**	**<15**	**15-30**	**30-45**	**>45**
<15	--	0.309	0.349	**0.001**	--	0.338	0.291	**0.005**
15-30	0	--	0.340	**0.001**	0	--	0.249	**0.003**
30-45	0	0	--	**0.002**	0	0.003	--	0.051
>45	**0.506**	**0.502**	**0.341**	--	**0.213**	**0.246**	0.086	--

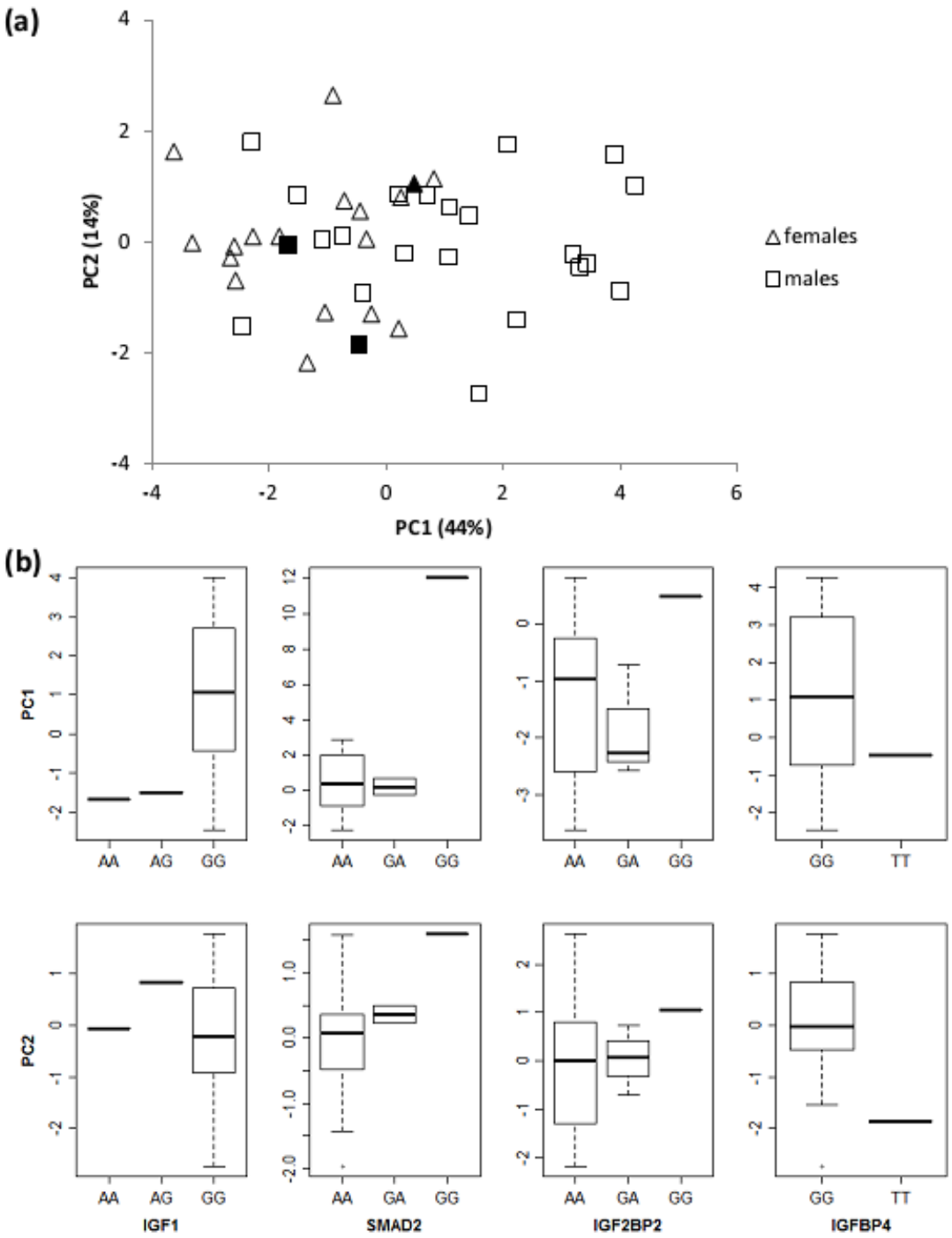
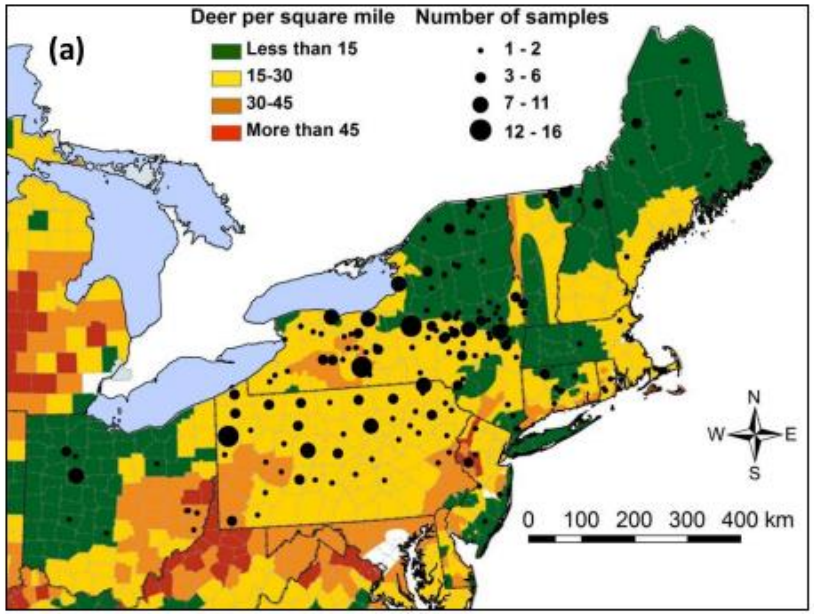
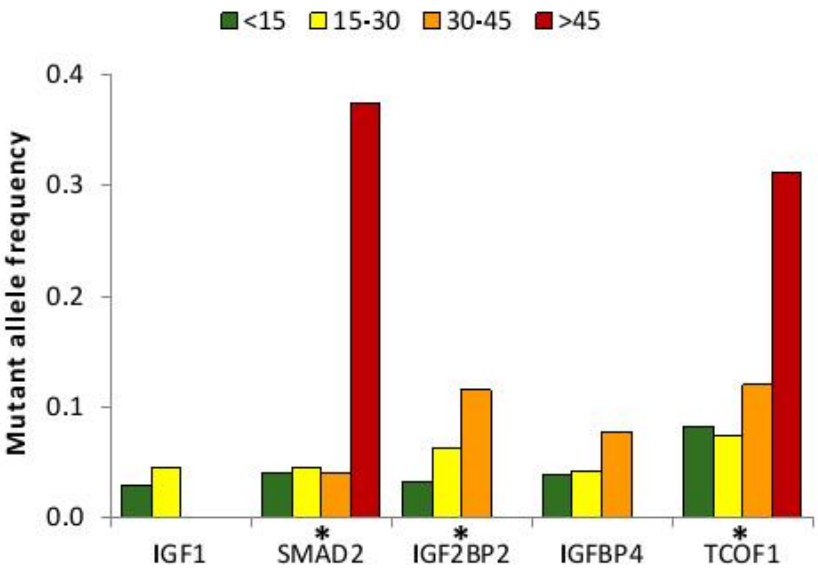
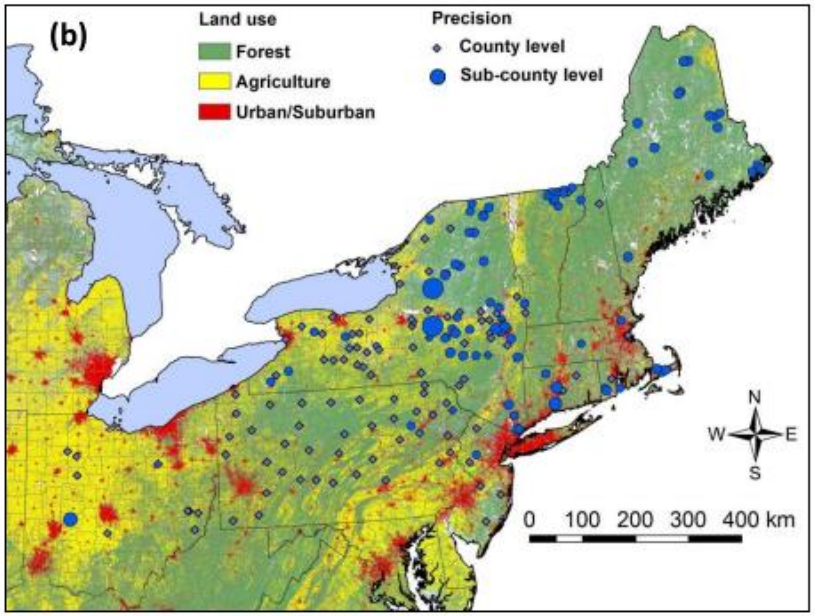


Figure 5.1. (a) Principal components analysis biplot of ten skull traits in 45 northeastern coyotes (25 males, 20 females). Two males and one female (in solid black symbols) on the periphery of their respective sex cluster were homozygous mutants. For clarity, the extreme outlier, suspected wolf zm14276, which is a homozygous mutant for *SMAD2*, is not shown. (b) Boxplots showing distribution of skull phenotypes in the first two principal components in relation to each SNPs genotype. For each SNP, only one individual was a homozygous mutant and is plotted as a horizontal bar against others of the same sex.


(a)
Deer per square mile
Less than 15
15-30
30-45
More than 45
Number of samples
1 - 2
3 - 6
7 - 11
12 - 16
N
W
E
S
0 100 200 300 400 km


<15
15-30
30-45
>45
Mutant allele frequency
0.4
0.3
0.2
0.1
0.0
IGF1
SMAD2
IGF2BP2
IGFBP4
TCOF1
*
*
*


(b)
Land use
Forest
Agriculture
Urban/Suburban
Precision
County level
Sub-county level
N
W
E
S
0 100 200 300 400 km

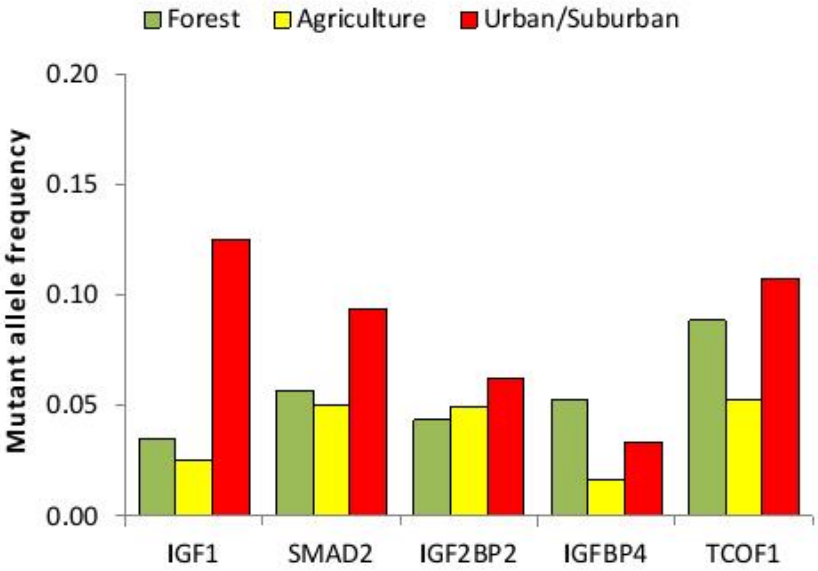

Forest
Agriculture
Urban/Suburban
Mutant allele frequency
0.20
0.15
0.10
0.05
0.00
IGF1
SMAD2
IGF2BP2
IGFBP4
TCOF1

Figure 5.2. Frequencies of mutant allele of five functional SNPs in wild coyotes grouped by (a) deer density and (b) human land use, with maps showing the locations of samples. Asterisk above gene name indicates non-independence of allele frequencies from ecological variable. Allele frequencies of *SMAD2*, *IGF2BP2*, and *TCOF1* were not independent of deer density. *P*-values for deer density G-tests: *IGF1* = 0.107, *SMAD2* = 0.001, *IGF2BP2* = 0.035, *IGFBP4* = 0.377, *TCOF1* = 0.042. *P*-values for land use G-tests: *IGF1* = 0.082, *SMAD2* = 0.163, *IGF2BP2* = 0.673, *IGFBP4* = 0.874, *TCOF1* = 0.377. In (b), size of circle is the buffer, drawn to scale, from which land use proportions were calculated for samples georeferenced with precision below the county level.

CHAPTER 6

GENERAL CONCLUSIONS AND OUTLOOK FOR FUTURE RESEARCH

Adaptive and neutral processes can create very similar genetic patterns, such as establishing locally distinct and differentiated populations. However, natural selection, being a non-random process, will be much more effective at creating the pattern more rapidly (Slatkin 1987). Genetic studies of natural populations have focused on neutral markers since the 1960s. The emphasis on neutral variation has allowed us to better understand the interactions of mutation, genetic drift, and migration, but has hindered our progress in understanding natural selection at the molecular level. For this reason, many molecular ecologists have advocated the shift away from neutral markers toward loci that reflect adaptive genetic variation (Morin *et al.* 2004; Kohn *et al.* 2006; Holderegger & Wagner 2008). SNPs are valuable markers because they can be used to detect population genetic structure and the genetic footprint of adaptation (Morin *et al*. 2004). A SNP analysis of genes that may affect fitness can illuminate the path from genetic differentiation to phenotypic (e.g., morphological or behavioral) differentiation among populations by highlighting loci that are subject to divergent selection. For these reasons, I took a spatially explicit genomics approach, utilizing SNPs to study hybridization, population genetic structure, and local adaptation in a uniquely large contemporary collection of an apex predator. The primary goal of this dissertation was to use genomic data to test the hypothesis that the variety of coyote in northeastern North America is an ecotype that has rapidly adapted, possibly through hybridization with wolves, to consume larger prey, namely white-tailed deer (Larivière & Crête 1993). I found evidence to support this hypothesis, but at an even finer spatial scale.

Admixed northeastern coyotes are genetically distinct from parental populations, and they occur in genetic populations or metapopulations that conform to the fine-grained habitat heterogeneity characteristic of the region. This heterogeneity corresponds to local variation in deer density and human land use.

The rapid establishment of the Coyote in the Northeast as the region's newest top predator and furbearer generated considerable interest among ecologists, wildlife managers, and the public (Parker 1995). However, prior to my study, only one region-wide genetic analysis of northeastern coyotes had been conducted (Kays *et al.* 2010), in which the authors looked at genetic variation in one locus: the mitochondrial control region, which is maternally inherited and thus limited in resolution. In Chapter 2, I conducted the first study of nuclear genetic variation and population structure in northeastern coyotes across the entire region. One of the aims was to evaluate the utility of SNPs ascertained from the dog genome as appropriate genetic markers for the estimation of genetic variability and population structure in a closely related wild canid. I found three primary genetic subdivisions across the region, and finer spatial structure when I zoomed in an area of recent contact between two colonization fronts. The data show that even sixteen biallelic SNPs with high heterozygosity genotyped in a dense sample are sufficient to resolve fine levels of population structure.

Hybridization may be a conduit for rapid evolutionary change, much of which may be adaptive. Northeastern coyotes may have acquired a suite of beneficial alleles from wolves and/or dogs that may have facilitated their expansion into forested and human-dominated environments. The hybrid nature of eastern coyotes has long been recognized (Lawrence & Bossert 1969; Silver & Silver 1969), but no one had evaluated the relative contributions of the various parental populations. In Chapter 3, I used ancestry-informative diagnostic SNPs to

estimate the relative contributions of western coyotes, western and eastern wolves, and domestic dogs to the admixed ancestry of Ohio and northeastern coyotes. I found that that all wild canids in the region are admixed, and on average about 63% coyote, 13% western wolf, 13% eastern wolf, and 11% dog, although there is some regional variation. Coyotes in Ohio had the highest proportion of western coyote ancestry, but it was still surprising to find substantial proportions of wolf DNA in Ohio, a state which has not seen wolves since they were exterminated in the 1800s. Eastern coyotes form an extensive and growing hybrid swarm. The extent of wolf introgression is expanding back westward into Ohio (this study) and Saskatchewan (Stronen *et al.* 2012), and southward into North Carolina (Bohling & Waits 2011). Wolf DNA, however, is not distributed randomly across habitat types. I found that canids in areas of high deer density are genetically more wolf-like, suggesting that natural selection for wolf-like traits may result in local adaptation at a fine geographic scale. Finally, my results demonstrate that interspecific sexual interactions among *Canis* are principally mediated by female coyotes mating with male wolves and dogs.

Population genetic structure is ubiquitous in nature, but genetically differentiated populations often take thousands of years to diverge. The results of Chapter 2 showed that northeastern coyotes occur in distinct populations. But, given that coyotes have colonized the region only in the last few decades and there are no obvious physical barriers to dispersal, it was puzzling how distinct populations of this highly mobile and generalist carnivore could be maintained. In Chapter 4, I examined how several ecological factors influence population genetic structure in northeastern coyotes. I found that human land use and deer density affect the distribution of genetic variation. These results are interesting because they show that ecology-induced genetic differences evolved within the last 30 to 80 years, as long as coyotes have inhabited the Northeast. The data also suggest that populations are becoming increasingly

divergent. Another intriguing finding is that northeastern coyotes appear to form a collection of genetically differentiated and overlapping metapopulations that conform to a mosaic of habitat types. I speculate that this unique genetic pattern is caused by the fine-grained habitat heterogeneity in the region, coupled with strong habitat preferences of individuals induced by early life experiences (Davis & Stamps 2004). Overall, the results from Chapter 4 demonstrate the instant formation of ecological barriers to gene flow following a rapid range and niche expansion.

The results from the previous chapters suggested that an adaptive process is occurring rapidly: northeastern coyote populations seem to be diverging and may be specializing to different habitat types and prey. For example, high deer density came out as an important factor in the spatial distribution of wolf alleles (Chapter 3) and in structuring genetic variation (Chapter 4). These results were consistent with the hypothesis that hybridization with wolves introduced genetic variation that enabled morphological adaptation for hunting deer (Kays *et al.* 2010). However, adaptation requires that natural selection act on ecologically and functionally important genes. In Chapter 5, I investigated the possibility of rapid morphological adaptation by genotyping five SNPs that are associated with body size and skull size, two important traits for any large mammalian predator. I found that allele frequencies in three of the five functional loci depended on deer density. Specifically, coyotes in the highest deer density habitats are highly differentiated from coyotes in the lower deer density habitats in respect to *SMAD2* and *TCOF1*, two of the functional genes. These results suggest that northeastern coyotes occur as locally adapted ecotypes. A substantial reduction of gene flow across habitats is apparently mediated by the density of white-tailed deer, a main prey species; and strong diversifying selection is acting on the genetic architecture that underlies morphological traits related to

predation. These functional genes had previously been studied in the domestic dog, but my study was the first spatially-explicit, population-level interrogation of these genes in a wild, non-model organism. I described the first documented evidence of any wild canids with homozygous mutant genotypes in these functional SNPs.

My research opens up additional questions. For example, *how did wolf-derived DNA arrive in Ohio?* I proposed three alternative hypotheses in Chapter 3 that may be tested with better sampling in Ohio and the Great Lakes region. *Is sex-biased hybridization in* Canis *mediated by differences in body size, social structure, or abundance? Is the apparent absence of hybridization between wild males and domestic females a real biological phenomenon, or an artifact of sampling bias possibly caused by maternal effects or the early extermination of hybrid pups?* Using Y-chromosome diagnostic markers and sampling rural and feral dogs as well as wild canids can address these questions. *How do individual coyotes perceive habitat quality and are they really dispersing to habitats similar to their natal one? Are parapatric populations in central New York becoming increasingly differentiated or increasingly homogeneous?* Detailed behavioral studies, such as with satellite telemetry, combined with long-term genetic studies are needed to tackle these questions. *What other morphological traits are undergoing rapid adaptation in northeastern coyotes?* More analyses of candidate genes of known function are very much needed to better understand the molecular basis of adaptation. Fortunately, much has been learned from the dog genome regarding the genetic basis of phenotypic diversity, disease, and behavior (Wayne & Ostrander 2007). The rich body of genomics research in domestic dogs enables interesting applications in ecological and evolutionary research of wild canids. Finally, *what is going with deer?* In Chapters 3-5, deer density was highlighted as an important ecological variable affecting the proportion of wolf alleles in individuals, the partitioning of

genetic variation, and the distribution of mutant alleles of morphology-associated genes. My studies depended on a somewhat crude binning of deer densities across the region and very few samples representing the highest density bin. More statistically robust regression analyses should be done using independent estimates of deer densities (as a continuous predictor variable) at a finer spatial scale with a more uniform sampling scheme across densities. Such analyses can aid in elucidating how prey mediate population differentiation and adaptation.

This dissertation represents the most extensive genomic investigation of northeastern coyotes in terms of sample size, number of molecular markers, and geographic representation. The results presented herein expand our understanding of the coyote in the eastern part of its range, but also of broad mechanisms underlying the rapid evolution of species. It is important to address the questions that remain outstanding through a holistic approach, not relying solely on molecular data in isolation of ecology and natural history, especially when we consider the reticulate evolutionary history of *Canis* in northeastern North America (Rutledge *et al.* 2012). In this way, not only will we better understand these enigmatic predators, but we will be better equipped to coexist with the closest wild relatives of our "best friend."

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APPENDIX A: SINGLE NUCLEOTIDE POLYMORPHISMS

SNPs genotyped in this dissertation. The rs identifier from the public database dbSNP (http://www.ncbi.nlm.nih.gov/snp), the chromosome and locus position from the 2005 CanFam2 dog genome assembly, the dissertation chapter, and the marker type is given for each of 108 SNPs.

dbSNP rs#	CanFam2 position	Chapter	Type
rs8795212	chr3:60134962	2	Hypervariable
rs24071674	chr4:33800600	2	Hypervariable
rs24241051	chr5:65740765	2	Hypervariable
rs24352476	chr6:17110138	2	Hypervariable
rs24514604	chr8:69650155	2	Hypervariable
rs22055760	chr10:45343436	2	Hypervariable
rs8946304	chr11:66863044	2	Hypervariable
rs22184574	chr12:17166054	2	Hypervariable
rs22603056	chr17:31508687	2	Hypervariable
rs22758397	chr19:50618604	2	Hypervariable
rs23209441	chr25:44793770	2	Hypervariable
rs23365246	chr27:5811313	2	Hypervariable
rs21988674	chr1:92426160	2, 4	Hypervariable
rs22444520	chr16:9533917	2, 4	Hypervariable
rs23051971	chr22:57259397	2, 4	Hypervariable
rs9205317	chr37:26421162	2, 4	Hypervariable
rs24175585	chr5:35001148	3, 4	Diagnostic
rs22333390	chr14:3736898	3, 4	Diagnostic
rs9150379	chr9:3587572	3, 4	Diagnostic
rs22877057	chr20:42599640	3, 4	Diagnostic
rs24471781	chr8:6763836	3, 4	Diagnostic
rs23367849	chr26:8080959	3, 4	Diagnostic
rs24514093	chr8:4937028	3, 4	Diagnostic
rs24543100	chr8:4551284	3, 4	Diagnostic
rs23617324	chr30:11604878	3, 4	Diagnostic
rs22161480	chr11:24081567	3, 4	Diagnostic
rs8612074	chr6:9043224	3, 4	Diagnostic
rs23909187	chr35:7375247	3, 4	Diagnostic
rs23278100	chr25:4634589	3, 4	Diagnostic
rs23037622	chr22:24036285	3, 4	Diagnostic
rs22928481	chr20:48770169	3, 4	Diagnostic
rs23050823	chr22:3943906	3, 4	Diagnostic
rs23006689	chr22:10587732	3, 4	Diagnostic
rs24517393	chr8:4989539	3, 4	Diagnostic
rs22645721	chr18:49329112	3, 4	Diagnostic

dbSNP rs#	CanFam2 position	Chapter	Type
rs22409691	chr15:31309035	3, 4	Diagnostic
rs23001750	chr22:11186281	3, 4	Diagnostic
rs22927609	chr20:7060544	3, 4	Diagnostic
rs22416514	chr15:6945840	3, 4	Diagnostic
rs23054155	chr22:9273100	3, 4	Diagnostic
rs22691222	chr9:3572341	3, 4	Diagnostic
rs22659787	chr9:3375420	3, 4	Diagnostic
rs22436136	chr15:7561843	3, 4	Diagnostic
rs22491491	chr16:9368697	3, 4	Diagnostic
rs22488932	chr16:8104516	3, 4	Diagnostic
rs22494347	chr16:43676315	3, 4	Diagnostic
rs22582321	chr17:8624315	3, 4	Diagnostic
rs9073720	chr17:39364422	3, 4	Diagnostic
rs24489243	chr8:4939363	3, 4	Diagnostic
rs24373496	chr7:9804508	3, 4	Diagnostic
rs24447332	chr7:4881807	3, 4	Diagnostic
rs9029227	chr29:16487251	3, 4	Diagnostic
rs24218607	chr5:81505841	3, 4	Diagnostic
rs23126832	chr23:35392765	3, 4	Diagnostic
rs8666298	chr4:13392059	3, 4	Diagnostic
rs23410089	chr27:26009285	3, 4	Diagnostic
rs22350704	chr15:34791197	3, 4	Diagnostic
rs23486713	chr29:6527627	3, 4	Diagnostic
rs22011433	chr1:17047825	3, 4	Diagnostic
rs23651611	chr30:26296172	3, 4	Diagnostic
rs24207725	chr5:29037489	3, 4	Diagnostic
rs21906101	chr1:69064725	3, 4	Diagnostic
rs22976400	chr21:7689005	3, 4	Diagnostic
rs21962387	chr1:22095829	3, 4	Diagnostic
rs22128776	chr11:66102349	3, 4	Diagnostic
rs21972855	chr1:20697427	3, 4	Diagnostic
rs24617980	chr9:41645497	3, 4	Diagnostic
rs23245491	chr25:36779413	3, 4	Diagnostic
rs23653965	chr30:11987344	3, 4	Diagnostic
rs22817050	chr2:44590374	3, 4	Diagnostic
rs22767921	chr2:57774505	3, 4	Diagnostic
rs23070823	chr23:11727587	3, 4	Diagnostic
rs24401025	chr7:56534713	3, 4	Diagnostic
rs8747831	chr17:57688984	3, 4	Diagnostic
rs24427396	chr7:42035045	3, 4	Diagnostic
rs22521423	chr16:57675695	3, 4	Diagnostic
rs24260906	chr6:44505782	3, 4	Diagnostic

dbSNP rs#	CanFam2 position	Chapter	Type
rs23966574	chr37:25655502	3, 4	Diagnostic
rs24312148	chr6:25401566	3, 4	Diagnostic
rs8982373	chr14:15009328	4	Hypervariable
rs8638173	chr8:70294996	4	Hypervariable
rs22023458	chr10:72019162	4	Hypervariable
rs24758998	chr13:55246440	4	Hypervariable
rs23016615	chr22:29442271	4	Hypervariable
rs24728016	chr12:11292298	4	Hypervariable
rs23430010	chr28:22397501	4	Hypervariable
rs21946549	chr1:58900092	4	Hypervariable
rs23986283	chr36:28858905	4	Hypervariable
rs23882163	chr34:21640421	4	Hypervariable
rs23249029	chr25:35378726	4	Hypervariable
rs23624964	chr30:18425833	4	Hypervariable
rs22854430	chr20:12532648	4	Hypervariable
rs23314486	chr26:40501752	4	Hypervariable
rs9013200	chr24:8356751	4	Hypervariable
rs9080222	chr17:42979944	4	Hypervariable
rs23689801	chr31:39352849	4	Hypervariable
rs9104999	chr11:75769588	4	Hypervariable
rs24031539	chr38:4798086	4	Hypervariable
rs23123984	chr23:16786463	4	Hypervariable
rs23987896	chr36:32465141	4	Hypervariable
rs23797773	chr33:19135556	4	Hypervariable
rs23188722	chr24:34119979	4	Hypervariable
rs23319896	chr26:19453763	4	Hypervariable
rs24564560	chr9:25422653	5	Functional
rs24098843	chr4:61932564	5	Functional
rs24445718	chr7:46696633	5	Functional
rs23872573	chr34:21414695	5	Functional
rs22437444	chr15:44231500	5	Functional

APPENDIX B: SPECIMENS

Specimens whose genetic profiles were analyzed. The New York State Museum specimen identifier, state or province, coordinates in decimal degrees, and method of SNP genotyping is given for each of 509 unique eastern coyotes (zm14276 and zm15083 were suspected wolves with an estimated coyote ancestry of 17% and 30%, respectively). The Roche high-resolution melting curve method is described in Chapter 2, and the Illumina GoldenGate method is described in Chapter 3.

Tissue ID	State/Province	Latitude	Longitude	Method
adk439	New York	43.93741496	-74.10040644	Illumina
adk483	New York	44.70434536	-73.9888616	Illumina
adk597	New York	44.69818744	-73.98173651	Illumina
adk2669	New York	43.88043882	-74.70805982	Roche
adk2706	New York	43.76892512	-75.03562171	Illumina
adk2796	New York	43.89472213	-74.68235374	Roche
adk2798	New York	43.90153901	-74.67316508	Illumina
adk2801	New York	43.90129677	-74.67346526	Illumina
adk2833	New York	43.93775995	-74.76875792	Illumina
adk2845	New York	42.79413159	-73.78896444	Illumina
adk2853	New York	43.7806245	-75.02236888	Roche, Illumina
adk2855	New York	43.77475934	-75.03653227	Roche
adk2868	New York	43.78931307	-75.02311786	Roche, Illumina
adk2880	New York	42.79608967	-73.78848604	Roche
mcz63139	Massachusetts	41.7307072	-70.474642	Roche
mcz63140	Massachusetts	41.7307072	-70.474642	Roche, Illumina
mcz63145	Massachusetts	41.687129	-70.270606	Roche, Illumina
mcz63343	Massachusetts	42.632525	-71.10982	Illumina
mcz63344	Massachusetts	41.696603	-70.384444	Roche, Illumina
mcz63683	Massachusetts	41.653054	-70.36686	Roche, Illumina
zm13499	New York	42.7075	-75.18806	Roche, Illumina
zm13514	New York	42.25	-73.5833	Illumina
zm13580	New York	42.7075	-75.18806	Roche, Illumina
zm13582	New York	42.7075	-75.18806	Roche
zm13615	New York	44.438048	-74.251511	Roche, Illumina
zm13616	New York	44.45343	-74.37572	Illumina
zm13642	New York	44.45343	-74.37572	Roche, Illumina
zm13652	New York	43.2678	-73.4287	Roche
zm13657	New York	43.2678	-73.4287	Roche, Illumina
zm13971	New York	42.7075	-75.18806	Roche, Illumina
zm13977	New York	42.7205	-73.8573	Roche
zm13979	New York	42.7075	-75.18806	Illumina
zm13982	New York	43.2678	-73.4287	Roche, Illumina
zm13984	New York	43.2678	-73.4287	Roche, Illumina
zm13986	New York	43.2678	-73.4287	Roche, Illumina
zm14206	New York	42.779322	-73.997531	Roche, Illumina
zm14207	New York	42.7052	-73.9035	Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zm14276	New York	43.227991	-74.056693	Roche, Illumina
zm14487	New York	43.135811	-73.874623	Roche, Illumina
zm14503	New York	43.135811	-73.874623	Illumina
zm14520	New York	42.934137	-74.115386	Roche
zm14522	New York	42.754	-73.934	Roche
zm14592	New York	42.753449	-73.724815	Roche
zm14595	New York	42.579983	-73.678142	Roche, Illumina
zm14623	New York	44.833333	-73.833333	Illumina
zm14624	New York	44.833333	-73.833333	Roche, Illumina
zm14625	New York	44.88755	-73.397409	Roche
zm14627	New York	44.733333	-75.266667	Roche, Illumina
zm14633	New York	44.932691	-74.216065	Illumina
zm14634	New York	44.932691	-74.216065	Illumina
zm14636	New York	44.932691	-74.216065	Illumina
zm14638	New York	44.805067	-74.290459	Illumina
zm14639	New York	44.932691	-74.216065	Illumina
zm14640	New York	44.932691	-74.216065	Illumina
zm14662	Vermont	43.926667	-72.671667	Roche
zm14663	Vermont	44.827403	-72.24437	Roche, Illumina
zm14665	Ohio	40.672553	-83.667515	Roche, Illumina
zm14666	Ohio	41.461747	-84.318357	Roche, Illumina
zm14667	Ohio	41.461747	-84.318357	Roche, Illumina
zm14671	Ohio	40.672553	-83.667515	Roche, Illumina
zm14672	Ohio	40.672553	-83.667515	Roche, Illumina
zm14673	Ohio	41.461747	-84.318357	Roche, Illumina
zm14674	Ohio	41.461747	-84.318357	Roche, Illumina
zm14700	Ohio	41.461747	-84.318357	Roche, Illumina
zm14701	Connecticut	41.595	-72.70667	Roche, Illumina
zm14702	Connecticut	41.913101	-72.989443	Roche, Illumina
zm14703	Connecticut	41.913101	-72.989443	Roche
zm14704	Connecticut	41.66111	-72.78	Roche, Illumina
zm14705	Connecticut	41.913101	-72.989443	Roche, Illumina
zm14706	Connecticut	41.3903	-72.86	Roche, Illumina
zm14708	Vermont	44.827403	-72.24437	Roche
zm14754	Vermont	44.827403	-72.24437	Roche, Illumina
zm14767	Vermont	44.827403	-72.24437	Roche
zm14769	New York	42.939353	-73.838391	Roche, Illumina
zm14780	Vermont	44.827403	-72.24437	Roche
zm14781	Vermont	44.827403	-72.24437	Roche, Illumina
zm14782	Ohio	40.672553	-83.667515	Roche, Illumina
zm14801	Ohio	40.672553	-83.667515	Illumina
zm14802	Vermont	44.827403	-72.24437	Roche
zm14807	Connecticut	41.812778	-72.31	Roche, Illumina
zm14817	Ohio	40.86222	-81.86194	Roche, Illumina
zm14818	Ohio	39.725864	-81.077399	Roche, Illumina
zm14819	Ohio	40.04929	-81.18505	Roche, Illumina
zm14820	Ohio	40.05131	-81.22302	Roche, Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zm14821	Ohio	40.012731	-80.995232	Roche
zm14822	Ohio	40.13378	-81.34681	Roche
zm14823	Ohio	40.012731	-80.995232	Roche, Illumina
zm14824	New York	42.754	-73.934	Roche
zm14826	Ohio	40.13378	-81.34681	Roche
zm14827	Ohio	39.92417	-83.80889	Roche, Illumina
zm14981	Vermont	44.911934	-71.915399	Roche, Illumina
zm15077	New York	42.881691	-77.473576	Roche, Illumina
zm15079	New York	42.665162	-77.770062	Roche
zm15082	New York	43.261583	-76.251276	Roche
zm15083	Vermont	44.95083	-72.30694	Illumina
zm15119	Vermont	44.827403	-72.24437	Roche
zm15120	New York	41.467753	-73.904014	Illumina
zm15121	New York	41.074499	-73.775179	Roche
zm15123	New York	41.181351	-73.729974	Roche
zm15124	New York	41.274455	-73.8107	Roche, Illumina
zm15126	New York	41.034685	-73.736235	Roche, Illumina
zm15128	Ohio	39.92417	-83.80889	Roche
zm15129	Ohio	40.69098	-80.951699	Roche
zm15130	Ohio	40.672553	-83.667515	Roche, Illumina
zm15131	Ohio	40.672553	-83.667515	Roche, Illumina
zm15132	Ohio	40.672553	-83.667515	Roche, Illumina
zm15133	Ohio	39.92417	-83.80889	Roche
zm15134	Ohio	40.672553	-83.667515	Roche
zm15135	Ohio	40.672553	-83.667515	Roche, Illumina
zm15136	Ohio	40.992966	-83.683031	Roche, Illumina
zm15137	Vermont	44.911934	-71.915399	Roche
zm15139	Vermont	44.911934	-71.915399	Illumina
zm15140	Vermont	44.911934	-71.915399	Roche
zm15142	Vermont	44.911934	-71.915399	Roche, Illumina
zm15143	Vermont	44.911934	-71.915399	Roche, Illumina
zm15144	Vermont	44.911934	-71.915399	Roche, Illumina
zm15145	Vermont	44.827403	-72.24437	Roche, Illumina
zm15146	Vermont	44.827403	-72.24437	Roche, Illumina
zm15147	Connecticut	41.595	-72.64583	Roche, Illumina
zm15148	Ohio	40.672553	-83.667515	Roche
zm15149	Ohio	40.672553	-83.667515	Roche, Illumina
zm15151	Vermont	43.141182	-73.271536	Roche, Illumina
zm15158	Vermont	43.141182	-73.271536	Roche
zm15159	Vermont	43.141182	-73.271536	Roche
zm15162	Vermont	43.141182	-73.271536	Roche
zm15166	Quebec	46.618889	-74.546389	Roche
zm15168	New Hampshire	44.642705	-71.207068	Roche
zm15171	New Hampshire	44.642705	-71.207068	Roche, Illumina
zm15172	New Hampshire	44.642705	-71.207068	Roche, Illumina
zm15173	New Hampshire	44.642705	-71.207068	Roche, Illumina
zm15175	New Hampshire	44.642705	-71.207068	Roche

Tissue ID	State/Province	Latitude	Longitude	Method
zm15176	New Hampshire	44.642705	-71.207068	Roche
zm15180	New Hampshire	44.642705	-71.207068	Roche, Illumina
zm15190	New Hampshire	44.642705	-71.207068	Roche
zm15193	New Hampshire	44.642705	-71.207068	Roche
zm15194	New Hampshire	44.642705	-71.207068	Roche, Illumina
zm15196	New Hampshire	44.642705	-71.207068	Roche
zm15197	New York	44.547502	-74.801027	Illumina
zm15198	New York	44.547502	-74.801027	Roche, Illumina
zm15199	New York	44.547502	-74.801027	Illumina
zm15285	New York	42.99806	-78.18778	Roche, Illumina
zm15286	New York	42.19333	-79.24889	Roche, Illumina
zm15287	New York	42.35611	-78.83194	Roche, Illumina
zm15292	Quebec	45.39083333	-72.037	Roche, Illumina
zm15293	Quebec	46.28333333	-71.35	Illumina
zm15305	Quebec	46.43566667	-71.0205	Illumina
zm15306	Quebec	46.804353	-71.177816	Illumina
zm15310	Quebec	46.53333333	-71.63333333	Illumina
zm15311	Quebec	46.568	-71.8355	Illumina
zm15313	Quebec	46.82233333	-70.3895	Illumina
zm15316	Quebec	46.36666667	-71.61666667	Illumina
zm15317	Quebec	45.39083333	-72.037	Roche, Illumina
zm15318	Quebec	46.82233333	-70.3895	Illumina
zm15319	Quebec	46.82233333	-70.3895	Illumina
zm15321	Quebec	47.05566667	-69.72366667	Illumina
zm15373	Quebec	46.82233333	-70.3895	Illumina
zm15374	Quebec	46.45983333	-71.526	Illumina
zm15376	Quebec	46.55733333	-71.43566667	Illumina
zm15377	Quebec	46.568	-71.8355	Illumina
zm15379	Quebec	46.23333333	-71.75	Roche, Illumina
zm15411	Quebec	47.43333333	-69.7	Illumina
zm15508	Quebec	46.14083333	-70.90483333	Illumina
zm15512	Quebec	46.36666667	-71.61666667	Illumina
zm15520	Quebec	46.2	-70.783413	Illumina
zm15525	New York	41.886714	-74.976887	Roche, Illumina
zm15526	New York	42.299242	-79.118138	Roche, Illumina
zm15531	New York	41.886714	-74.976887	Illumina
zm15536	New York	41.886714	-74.976887	Roche, Illumina
zm15537	New York	41.886714	-74.976887	Roche, Illumina
zm15541	Ohio	39.687369	-82.991969	Roche, Illumina
zm15544	New York	41.886714	-74.976887	Roche, Illumina
zm15570	New York	43.032424	-73.93412	Roche
zm15575	New Jersey	39.966934	-74.308535	Roche
zm15576	New Jersey	39.966934	-74.308535	Roche, Illumina
zm15582	New Jersey	40.683863	-74.753411	Roche, Illumina
zm15583	New Jersey	40.683863	-74.753411	Roche, Illumina
zm15584	New Jersey	39.536561	-74.690225	Roche, Illumina
zm15585	New Jersey	41.128848	-74.687549	Roche, Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zm15586	New Jersey	39.536561	-74.690225	Roche, Illumina
zm15588	New Jersey	40.964675	-74.267253	Roche
zm15589	New Jersey	40.1416398	-74.7306135	Illumina
zm15590	New Jersey	41.128848	-74.687549	Illumina
zm15609	New Jersey	40.566022	-74.919654	Roche, Illumina
zm15610	New Jersey	40.566022	-74.919654	Roche, Illumina
zm15611	New Jersey	40.566022	-74.919654	Roche, Illumina
zm15612	New Jersey	40.566022	-74.919654	Roche, Illumina
zm15613	New Jersey	40.566022	-74.919654	Roche, Illumina
zm15614	New Jersey	40.566022	-74.919654	Roche, Illumina
zm15615	Pennsylvania	41.783562	-77.912106	Illumina
zm15616	Pennsylvania	41.33215	-77.019737	Roche, Illumina
zm15617	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zm15618	Pennsylvania	41.680868	-80.068469	Roche
zm15619	Pennsylvania	41.792053	-77.25374	Roche, Illumina
zm15620	Pennsylvania	42.004316	-80.068469	Roche, Illumina
zm15621	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zm15622	Pennsylvania	40.392431	-76.80982	Roche, Illumina
zm15623	Pennsylvania	41.814024	-75.788693	Roche, Illumina
zm15624	Pennsylvania	41.792053	-77.25374	Roche, Illumina
zm15625	Pennsylvania	41.76406	-76.471893	Roche, Illumina
zm15626	Pennsylvania	41.516888	-75.989637	Roche, Illumina
zm15627	Pennsylvania	41.516888	-75.989637	Roche, Illumina
zm15628	Pennsylvania	40.908869	-77.808908	Roche, Illumina
zm15629	Pennsylvania	41.608559	-75.296495	Roche, Illumina
zm15635	Maine	45.893547	-69.990258	Illumina
zm15637	Maine	45.254268	-70.258498	Illumina
zm15652	Maine	44.830191	-67.332561	Illumina
zm15661	Maine	44.761704	-67.261568	Illumina
zm15669	Maine	46.78867	-68.610528	Illumina
zm15679	Maine	46.783367	-68.499135	Illumina
zm15681	Maine	46.262019	-68.875396	Illumina
zm15682	Maine	44.736321	-67.481982	Illumina
zm15686	Maine	44.830191	-67.332561	Illumina
zm15688	Maine	45.893547	-69.990258	Illumina
zm15689	Maine	45.812965	-67.973783	Illumina
zm15691	Maine	45.774935	-68.117878	Illumina
zm15692	Maine	45.584535	-68.097409	Illumina
zm15705	Maine	45.893547	-69.990258	Illumina
zm15711	Maine	45.436914	-69.6821	Illumina
zm15715	Maine	45.803316	-68.239401	Illumina
zm15721	Maine	46.29083	-68.829082	Illumina
zm15724	Maine	43.680616	-70.727366	Illumina
zm15729	Maine	44.830191	-67.332561	Illumina
zm15753	Maine	44.8167686	-68.5111771	Illumina
zm15861	Rhode Island	41.53046	-71.700365	Illumina
zm15884	Rhode Island	41.673491	-71.524881	Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zm15951	Massachusetts	42.364619	-71.005515	Illumina
zm15952	Rhode Island	41.493326	-71.668087	Illumina
zm15953	Massachusetts	42.338635	-72.095355	Illumina
zm15973	Pennsylvania	41.432665	-75.631598	Illumina
zm15974	Pennsylvania	41.432665	-75.631598	Illumina
zm15976	Pennsylvania	41.147462	-75.969372	Illumina
zm15977	Pennsylvania	41.2822	-76.145015	Illumina
zm15978	Pennsylvania	41.432665	-75.631598	Illumina
zm15979	Pennsylvania	41.432665	-75.631598	Illumina
zm15980	Pennsylvania	41.147462	-75.969372	Illumina
zm15981	Pennsylvania	41.475927	-75.182098	Illumina
zm15985	Rhode Island	41.497105	-71.367396	Illumina
zm15987	Pennsylvania	40.608019	-75.590547	Illumina
zt1	Vermont	44.867161	-72.26096	Roche, Illumina
zt2	Vermont	44.983534	-71.79439	Roche, Illumina
zt3	Vermont	44.802368	-72.280098	Roche, Illumina
zt4	Vermont	44.867161	-72.26096	Roche, Illumina
zt5	Vermont	44.911789	-72.016683	Roche, Illumina
zt6	Vermont	44.707634	-72.18894	Roche, Illumina
zt7	Vermont	44.9602	-71.99982	Roche, Illumina
zt9	Vermont	44.983534	-71.79439	Roche, Illumina
zt10	Vermont	44.802368	-72.280098	Roche
zt11	Vermont	44.753679	-71.63029	Roche, Illumina
zt12	Vermont	44.911789	-72.016683	Roche, Illumina
zt13	Vermont	44.903998	-72.405752	Roche, Illumina
zt14	Vermont	44.911789	-72.016683	Roche
zt19	Vermont	44.93224	-72.21889	Illumina
zt40	New York	43.044879	-74.858965	Roche, Illumina
zt41	New York	42.743931	-75.546151	Roche, Illumina
zt43	New York	43.043429	-74.858993	Roche, Illumina
zt140	New York	41.041034	-73.78789	Illumina
zt142	Rhode Island	41.493497	-71.377605	Roche
zt148	Rhode Island	41.520668	-71.288018	Roche
zt163	New York	42.96746866	-75.91561672	Roche
zt165	New York	42.96746866	-75.91561672	Roche
zt168	New York	42.96746866	-75.91561672	Roche
zt169	New York	42.96746866	-75.91561672	Illumina
zt170	New York	42.96746866	-75.91561672	Roche, Illumina
zt171	New York	42.96746866	-75.91561672	Roche, Illumina
zt172	New York	42.96746866	-75.91561672	Illumina
zt173	New York	42.96746866	-75.91561672	Roche, Illumina
zt174	New York	42.96746866	-75.91561672	Illumina
zt175	New York	42.96746866	-75.91561672	Roche, Illumina
zt176	New York	42.96746866	-75.91561672	Illumina
zt177	New York	42.96746866	-75.91561672	Roche, Illumina
zt178	New York	42.96746866	-75.91561672	Illumina
zt179	New York	42.96746866	-75.91561672	Roche, Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zt181	New York	42.96746866	-75.91561672	Illumina
zt182	New York	42.96746866	-75.91561672	Roche, Illumina
zt183	New York	42.96746866	-75.91561672	Roche, Illumina
zt184	New York	42.96746866	-75.91561672	Roche, Illumina
zt185	New York	42.96746866	-75.91561672	Roche
zt186	New York	42.96746866	-75.91561672	Roche
zt187	New York	42.96746866	-75.91561672	Roche
zt188	New York	42.93419341	-75.4445842	Roche, Illumina
zt190	New York	42.93419341	-75.4445842	Roche, Illumina
zt192	New York	42.93419341	-75.4445842	Roche, Illumina
zt194	New York	42.93419341	-75.4445842	Roche, Illumina
zt196	New York	42.93419341	-75.4445842	Roche, Illumina
zt200	New York	42.93419341	-75.4445842	Roche, Illumina
zt201	New York	42.93419341	-75.4445842	Roche
zt203	New York	42.93419341	-75.4445842	Roche
zt206	New York	43.11762363	-74.78627634	Roche, Illumina
zt210	New York	43.11762363	-74.78627634	Roche, Illumina
zt213	New York	43.06692343	-75.08404586	Roche, Illumina
zt220	New York	43.06692343	-75.08404586	Roche, Illumina
zt221	New York	43.06692343	-75.08404586	Roche, Illumina
zt222	New York	43.04363486	-75.87392558	Roche, Illumina
zt225	New York	42.85086864	-75.34812946	Illumina
zt226	New York	42.60256014	-75.93606119	Roche, Illumina
zt227	New York	42.93756976	-74.29816992	Roche, Illumina
zt228	New York	42.93756976	-74.29816992	Illumina
zt232	New York	42.93756976	-74.29816992	Roche, Illumina
zt234	New York	43.09951294	-74.300624	Illumina
zt238	New York	42.47442084	-73.77329947	Roche, Illumina
zt239	New York	42.47442084	-73.77329947	Roche, Illumina
zt240	New York	42.47442084	-73.77329947	Roche, Illumina
zt241	New York	42.47442084	-73.77329947	Roche, Illumina
zt242	New York	43.20654342	-75.51850882	Roche, Illumina
zt244	New York	43.20654342	-75.51850882	Roche, Illumina
zt246	New York	43.56991161	-75.35532681	Illumina
zt247	New York	43.08709609	-75.9138091	Roche, Illumina
zt249	New York	43.08709609	-75.9138091	Roche, Illumina
zt250	Pennsylvania	41.94859006	-75.75648635	Roche, Illumina
zt251	Pennsylvania	41.94859006	-75.75648635	Roche, Illumina
zt252	Pennsylvania	41.94859006	-75.75648635	Roche
zt253	Pennsylvania	41.94859006	-75.75648635	Roche, Illumina
zt254	Pennsylvania	41.94859006	-75.75648635	Roche, Illumina
zt258	New York	42.9831072	-73.27525674	Roche, Illumina
zt260	New York	43.22796542	-77.74361085	Roche, Illumina
zt261	New York	43.22796542	-77.74361085	Illumina
zt262	New York	43.22796542	-77.74361085	Roche, Illumina
zt263	New York	43.22796542	-77.74361085	Illumina
zt264	New York	43.22796542	-77.74361085	Roche, Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zt267	New York	43.22796542	-77.74361085	Roche, Illumina
zt268	New York	43.22796542	-77.74361085	Illumina
zt269	New York	43.22796542	-77.74361085	Roche
zt273	New York	41.98746685	-74.84234228	Roche, Illumina
zt275	New York	41.98746685	-74.84234228	Roche, Illumina
zt280	New York	43.70031131	-76.10994938	Illumina
zt281	New York	43.70031131	-76.10994938	Illumina
zt282	New York	43.70031131	-76.10994938	Illumina
zt284	New York	43.70031131	-76.10994938	Illumina
zt285	New York	43.70031131	-76.10994938	Illumina
zt288	New York	43.70031131	-76.10994938	Illumina
zt289	New York	43.70031131	-76.10994938	Illumina
zt290	New York	43.70031131	-76.10994938	Roche, Illumina
zt292	New York	44.42203264	-75.42543637	Illumina
zt293	New York	44.42203264	-75.42543637	Illumina
zt295	New York	43.86620199	-75.40609502	Illumina
zt298	New York	43.86620199	-75.40609502	Illumina
zt299	New York	43.14440967	-76.88835139	Roche, Illumina
zt300	New York	43.14440967	-76.88835139	Roche, Illumina
zt301	New York	43.14440967	-76.88835139	Illumina
zt302	New York	43.14440967	-76.88835139	Roche, Illumina
zt303	New York	42.91871212	-77.14230345	Roche, Illumina
zt305	New York	42.91871212	-77.14230345	Roche
zt306	New York	42.91871212	-77.14230345	Illumina
zt307	New York	42.91871212	-77.14230345	Roche
zt309	New York	42.91871212	-77.14230345	Roche
zt310	New York	42.91871212	-77.14230345	Roche
zt311	New York	42.91871212	-77.14230345	Roche
zt312	New York	42.91871212	-77.14230345	Roche
zt314	New York	42.68019591	-76.77428013	Roche
zt315	New York	42.58792252	-77.00319371	Roche
zt317	New York	42.38877348	-77.24356045	Roche, Illumina
zt318	New York	42.38877348	-77.24356045	Roche, Illumina
zt319	New York	42.38877348	-77.24356045	Roche, Illumina
zt320	New York	42.38877348	-77.24356045	Roche, Illumina
zt323	New York	42.51579496	-78.00566688	Roche, Illumina
zt324	New York	42.51579496	-78.00566688	Illumina
zt325	New York	42.51579496	-78.00566688	Roche, Illumina
zt326	New York	43.14440967	-76.88835139	Roche, Illumina
zt327	New York	43.14440967	-76.88835139	Roche, Illumina
zt329	New York	43.14440967	-76.88835139	Roche, Illumina
zt330	New York	43.14440967	-76.88835139	Roche, Illumina
zt331	New York	43.14440967	-76.88835139	Roche, Illumina
zt332	New York	42.68416524	-77.22218042	Roche, Illumina
zt333	New York	42.2052702	-76.955346	Roche, Illumina
zt334	New York	42.4974676	-77.54640004	Roche, Illumina
zt336	New York	43.10148645	-78.46485445	Roche

Tissue ID	State/Province	Latitude	Longitude	Method
zt337	New York	42.95534491	-78.00289656	Roche, Illumina
zt338	New York	42.6774954	-77.40583264	Roche, Illumina
zt339	New York	42.49493355	-77.7814625	Roche
zt341	New York	42.49493355	-77.7814625	Roche, Illumina
zt342	New York	42.49493355	-77.7814625	Roche, Illumina
zt347	New York	42.61935105	-76.72432219	Roche
zt348	New York	42.61935105	-76.72432219	Roche
zt349	New York	42.61935105	-76.72432219	Roche
zt350	New York	42.61935105	-76.72432219	Roche
zt353	New York	42.727	-75.1485	Roche
zt357	New York	42.727	-75.1485	Roche
zt360	New York	42.727	-75.1485	Roche, Illumina
zt361	New York	42.727	-75.1485	Roche, Illumina
zt362	New York	42.727	-75.1485	Roche, Illumina
zt369	New York	42.70841	-74.14649	Roche
zt380	New York	42.857	-74.9973	Roche, Illumina
zt381	New York	42.777	-74.928	Roche, Illumina
zt382	New York	42.857	-74.9973	Roche, Illumina
zt383	New York	42.344	-74.2452	Roche, Illumina
zt384	New York	42.815	-74.5968	Roche, Illumina
zt385	New York	42.815	-74.5968	Roche, Illumina
zt387	New York	42.815	-74.5968	Roche, Illumina
zt388	New York	42.815	-74.5968	Roche, Illumina
zt389	New York	42.815	-74.5968	Roche, Illumina
zt390	New York	42.815	-74.5968	Illumina
zt391	New York	42.815	-74.5968	Roche, Illumina
zt393	New York	42.777	-74.928	Roche, Illumina
zt394	New York	42.777	-74.928	Roche, Illumina
zt395	New York	42.457	-75.1025	Roche, Illumina
zt396	New York	42.457	-75.1025	Roche, Illumina
zt399	New York	42.359	-74.5205	Roche, Illumina
zt400	New York	42.359	-74.5205	Roche, Illumina
zt401	New York	42.384	-74.8129	Roche, Illumina
zt402	New York	42.384	-74.8129	Roche, Illumina
zt403	New York	42.384	-74.8129	Roche, Illumina
zt404	New York	42.344	-77.1237	Roche, Illumina
zt406	New York	42.344	-77.1237	Roche, Illumina
zt409	New York	42.344	-77.1237	Roche, Illumina
zt410	New York	42.344	-77.1237	Roche, Illumina
zt411	New York	42.344	-77.1237	Roche, Illumina
zt412	New York	42.344	-77.1237	Roche, Illumina
zt413	New York	42.344	-77.1237	Roche, Illumina
zt414	New York	42.344	-77.1237	Roche, Illumina
zt415	New York	42.344	-77.1237	Roche, Illumina
zt416	New York	42.344	-77.1237	Roche, Illumina
zt417	New York	42.344	-77.1237	Roche, Illumina
zt418	New York	42.344	-77.1237	Roche, Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zt419	New York	42.344	-77.1237	Roche, Illumina
zt421	New York	42.712955	-73.878361	Roche, Illumina
zt446	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt449	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt450	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt451	Pennsylvania	40.829959	-79.423361	Roche, Illumina
zt452	Pennsylvania	40.908869	-77.808908	Roche, Illumina
zt454	Pennsylvania	41.792053	-77.25374	Roche, Illumina
zt455	Pennsylvania	41.418126	-78.660232	Roche, Illumina
zt456	Pennsylvania	41.232543	-77.652679	Roche, Illumina
zt457	Pennsylvania	41.418126	-78.660232	Roche, Illumina
zt458	Pennsylvania	41.127334	-79.002202	Roche, Illumina
zt459	Pennsylvania	41.127334	-79.002202	Roche, Illumina
zt460	Pennsylvania	42.004316	-80.068469	Roche, Illumina
zt462	Pennsylvania	40.769358	-77.060296	Roche, Illumina
zt463	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt465	Pennsylvania	41.94859006	-75.75648635	Roche, Illumina
zt466	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt468	Pennsylvania	40.506598	-78.701545	Roche, Illumina
zt469	Pennsylvania	40.530297	-77.447552	Roche, Illumina
zt471	Pennsylvania	41.76406	-76.471893	Roche, Illumina
zt472	Pennsylvania	41.76406	-76.471893	Roche, Illumina
zt475	Pennsylvania	41.76406	-76.471893	Roche, Illumina
zt476	Pennsylvania	41.76406	-76.471893	Roche, Illumina
zt478	Pennsylvania	41.418126	-78.660232	Roche, Illumina
zt479	Pennsylvania	41.805013	-79.294353	Roche, Illumina
zt480	Pennsylvania	40.506598	-78.701545	Roche, Illumina
zt481	Pennsylvania	41.800266	-77.902493	Roche
zt482	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt484	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt486	Pennsylvania	41.805013	-79.294353	Illumina
zt492	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt600	Pennsylvania	40.992599	-78.469614	Roche, Illumina
zt601	Pennsylvania	40.413412	-77.979075	Roche, Illumina
zt602	Pennsylvania	40.413412	-77.979075	Roche, Illumina
zt603	Pennsylvania	40.908869	-77.808908	Roche, Illumina
zt604	Pennsylvania	39.854611	-80.230327	Roche, Illumina
zt605	Pennsylvania	39.854611	-80.230327	Roche, Illumina
zt606	Pennsylvania	41.804634	-78.565332	Roche, Illumina
zt607	Pennsylvania	41.804634	-78.565332	Roche, Illumina
zt608	Pennsylvania	41.804634	-78.565332	Roche, Illumina
zt609	Pennsylvania	39.854611	-80.230327	Roche, Illumina
zt610	Pennsylvania	39.854611	-80.230327	Roche, Illumina
zt614	Pennsylvania	41.053932	-75.34324	Roche, Illumina
zt623	Pennsylvania	41.383852	-79.732481	Roche, Illumina
zt625	Pennsylvania	41.680868	-80.068469	Roche, Illumina
zt627	Pennsylvania	40.988623	-80.336807	Roche, Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
zt629	Pennsylvania	39.935608	-79.657802	Roche, Illumina
zt630	Pennsylvania	40.7658	-75.324697	Illumina
zt631	Pennsylvania	41.418126	-78.660232	Roche, Illumina
zt632	Pennsylvania	39.935608	-79.657802	Roche, Illumina
zt633	Pennsylvania	40.908869	-77.808908	Roche, Illumina
zt634	Pennsylvania	40.506598	-78.701545	Roche, Illumina
zt636	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt637	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt638	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt639	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt640	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt641	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt642	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt643	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt646	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt650	Pennsylvania	40.6520	-79.0880	Roche, Illumina
zt651	Pennsylvania	40.506598	-78.701545	Roche, Illumina
zt653	Pennsylvania	40.3110	-79.4670	Roche, Illumina
zt657	Pennsylvania	41.061168	-76.422792	Roche, Illumina
zt658	Pennsylvania	40.482	-78.349	Roche
zt660	Pennsylvania	42.004316	-80.068469	Roche, Illumina
zt661	Pennsylvania	42.004316	-80.068469	Roche, Illumina
zt671	Pennsylvania	40.908869	-77.808908	Roche, Illumina
zt672	Pennsylvania	41.792053	-77.25374	Roche, Illumina
zt675	Pennsylvania	41.418126	-78.660232	Roche, Illumina
zt676	Pennsylvania	40.992599	-78.469614	Illumina
zt677	Pennsylvania	41.805013	-79.294353	Roche, Illumina
zt678	Pennsylvania	40.392431	-76.80982	Roche, Illumina
zt679	Pennsylvania	41.427386	-76.517451	Roche, Illumina
zt690	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt691	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt692	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt693	Pennsylvania	41.296773	-80.2423	Roche, Illumina
zt694	Pennsylvania	41.680868	-80.068469	Roche, Illumina
zt695	Pennsylvania	41.680868	-80.068469	Roche, Illumina
zt696	Pennsylvania	41.94859006	-75.75648635	Roche
zt697	Pennsylvania	41.94859006	-75.75648635	Roche, Illumina
zt698	Pennsylvania	41.94859006	-75.75648635	Roche, Illumina
zt699	Pennsylvania	41.33215	-77.019737	Roche, Illumina
zt700	Pennsylvania	41.33215	-77.019737	Roche, Illumina
zt701	Pennsylvania	41.33215	-77.019737	Roche, Illumina
zt702	Pennsylvania	41.33215	-77.019737	Illumina
zt703	Pennsylvania	41.33215	-77.019737	Illumina
zt704	Pennsylvania	41.33215	-77.019737	Roche, Illumina
ztpb2	New York	42.712955	-73.878361	Illumina
ztpb6	New York	42.712955	-73.878361	Illumina
ztpb10	New York	42.712955	-73.878361	Illumina

Tissue ID	State/Province	Latitude	Longitude	Method
ztpb13	New York	42.712955	-73.878361	Illumina
ztpb150664	New York	42.712955	-73.878361	Illumina