GENETIC STATUS AND MORPHOLOGICAL CHARACTERISTICS OF MAINE COYOTES AS RELATED TO NEIGHBORING COYOTE AND WOLF POPULATIONS[1]

PAUL J. WILSON, Natural Resources DNA Profiling & Forensic Center, Department of Biology, Trent University, 1600 East Bank Drive, Peterborough, Ontario, K9J 7B8
WALTER J. JAKUBAS, Maine Department of Inland Fisheries and Wildlife, Bangor, Maine, 04444
SHEVENELL MULLEN, Department of Wildlife Ecology, University of Maine, Orono, Maine, 04473

Summary

This project was undertaken in response to discussions on wolf recovery in the Northeast and how hybridization with coyotes might affect the feasibility of wolf recovery, the ecological justification for wolf recovery, and coyote management. The original objectives of the study were to (1) characterize the types of *Canis* in Maine – i.e. coyotes, eastern Canadian wolves, gray wolves, or hybrids; (2) determine the geographic origin of these canids; and (3) locate historic specimens of New England wolves and determine their genetic profile. In addition to these objectives, we tested the hypothesis that wolf genes have not introgressed into the eastern coyote population by comparing the genetic profiles of 100 coyotes collected from Maine to wolves from Quebec and Ontario; eastern coyotes from New York and New Brunswick; and western coyotes from

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Texas, Ohio, and North Carolina. Based on Bayesian cluster analysis and estimates of ancestry, 93% (n = 100) of Maine's canids had ancestries > 50% eastern coyote, 22% had a wolf ancestries >5%, one animal had a wolf ancestry of 89%, and only 4% of Maine coyotes had ancestries similar to western coyotes (i.e., >50% western coyote). The genetic structure of coyote populations from Maine, New York, and New Brunswick were closely related based on measures of genetic distance (F_{ST} estimates, Nei's genetic distance measure). These coyote populations, in turn, shared similar genetic ancestries, which included hybridization with eastern Canadian wolves (Bayesian cluster analysis). Finally, these eastern coyote populations showed a degree of genetic overlap with eastern Canadian wolves (Principle Component Analysis) that was consistent with a *C. latrans* x *lycaon* mixture. Based upon these results, we reject the hypothesis that wolf genes have not introgressed into the eastern coyote population. These eastern coyote populations showed significant differentiation from canid populations from Quebec, and Algonquin Provincial Park (Ontario) indicating low levels of gene flow between these regions. There were limitations to using genetic profiles to differentiate eastern Canadian wolves from eastern coyotes, because both canids have composite hybrid genomes. These limitations extended into the phenotype of the animals. One Maine animal, with an ancestry of 89% eastern Canadian wolf, was one of the smallest canids (12.3 kg [27.0 lb] adult female). Canids having small body statures (<18.1 kg [40.0 lb]) and having various amounts of eastern Canadian wolf ancestry were also identified from specimens collected in Algonquin Park. Discriminant function analysis identified a set of six morphometrical variables that could be used to assign canid specimens (79.6% accuracy) to their correct population (i.e., Quebec wolf, Quebec coyote, or Maine coyote), and suggested that

Quebec wolves and coyotes may be hybridizing. Challenges that wildlife agencies face in dealing with a hybrid coyote population include developing workable standards for identifying canids in the Northeast, determining the degree of protection that can be given to wolves in a hybrid zone, and devising management plans that will provide that protection.

Introduction

At the time Europeans first colonized North America, eastern timber wolves (*Canis lycaon* and possibly *Canis lupus*) occupied much of what is now the northeastern United States and eastern Canada (Nowak 1995, Wilson et al. 2003), while coyotes (*Canis latrans*) ranged from north central Mexico, through the central prairie region of the United States, to south central Canada (Parker 1995). Over the past 150 years, the coyote expanded its geographic range over North America in response to human activities and to the reduction of wolf numbers throughout the U.S. and Canada (Wayne et al. 1992, Moore and Parker 1992, Parker 1995). In the Northeast, following a 40-year period during which few, if any, large wild canids were known to occur in New York, a coyote-like animal was reported in the St. Lawrence Valley area in 1920. Reports of large coyote-like animals continued to increase in the early 1930s in Ontario, and were considered to be common in the Adirondacks of New York during the 1950s. In Maine, coyotes were noticed as early as 1936; however, it wasn't until the 1960's that people perceived that the coyote population was rapidly increasing (Richens and Hugie 1974, Parker 1995). By the 1970s, these animals had extended their range across southeastern Canada reaching Newfoundland in 1987 (Moore and Parker 1992).

This large coyote-like canid, described as the “eastern coyote”, is considered intermediate between western coyotes and gray wolves (*Canis lupus*) in body size and skull characteristics (Gaskin 1975, Lawrence and Bossart 1975, Nowak 1979). In Maine, the skulls of eastern coyotes average 6% - 11% larger than skulls of western coyotes (Hilton 1978) and the average coyote weighs approximately 4.5 kg (10 lb) more then the average western coyote (Richens and Hugie 1974, Parker 1995). Although the morphology of eastern coyotes may differ from western coyotes, it is less clear whether the two populations have distinct behavioral differences. Eastern coyotes exhibited less aggression towards each other than western coyotes, in studies of captive coyotes (Silver and Silver 1969), but they did not determine whether higher intraspecific aggression affected the ability of western coyotes to hunt cooperatively. Eastern coyotes prey more frequently on large prey (e.g., white-tailed deer [*Odocoileus virginianus*]) than western coyotes, with white-tailed deer comprising about 60% of their winter diet (Messier et al. 1986, Litvaitis and Harrison 1989, Parker 1995). However, the prevalence of large prey in the diets of eastern coyotes may have more to do with prey availability and vulnerability (e.g., deer in deep snow) than behavioral differences between eastern and western coyotes. In western habitats where deer are common, coyotes also prey on deer, and like their eastern counterparts, may hunt cooperatively with two or more individuals (Bowen 1981, Gese and Grothe 1995).

The size difference between eastern and western coyotes was noticed soon after coyotes first appeared in the east (Hilton 1978, Parker 1995), and speculation was common that the large size of the eastern coyote was the result of hybridization with wolves or domestic dogs (Silver and Silver 1969, Hilton 1978). Lawrence and Bossert

(1969, as cited in Hilton 1978) concluded, based on morphological characteristics, that the physical form of eastern coyotes was the likely the result of hybridization with either wolves or dogs. Early studies on wolves hybridizing with eastern coyotes implied a degree of genetic mixing between coyotes and wolves which may have occurred during the colonization process from Minnesota or Manitoba (Parker 1995).

Alternatively, Thurber and Peterson (1991), hypothesized that increased food supply alone, even without genetic selection, may account for the larger size of eastern coyotes. They reasoned that if hybridization was responsible for the large size of the eastern coyote, then coyotes in New England should be smaller then coyotes in Minnesota, since the nearest wolves New England coyotes could mate with were the small Algonquin wolves. Finally, Schmitz and Lavigne (1987) hypothesized that prey size and genetic selection favor larger coyotes. These authors present evidence that wolf size decreased in central Ontario at the same time that coyote size increased. They attribute this change in size to wolves preying on smaller prey over time (diet changed from moose and caribou to deer) and coyotes preying on larger animals (more deer, as deer became abundant in this area). They did not address the possibility that this convergence in size between the two species may be due to hybridization.

Genetic analyses of wolves in Minnesota and eastern Canada (Lehman et al. 1991, Wayne and Lehman 1992, Roy et al. 1994) indicated that wolves and coyotes hybridized in these regions. Although these studies indicated the presence of coyote genes in some wolf populations, they presented no evidence that wolf genes introgressed into the coyote population (Roy et al. 1994, Lehman et al. 1991, Pilgrim et al. 1998). Biologists speculated that when male wolves mated opportunistically with female coyotes, the offspring from these matings would only form packs with wolves. Thus, wolf-coyote matings were believed to result in coyote genes being passed into the wolf population, but not wolf genes being passed back into the coyote population. Roy et al. (1994) using nuclear DNA analyses, and 18 tissue samples from Maine coyotes, reported that coyotes were genetically similar across North America.

Additional light was shed on the question of whether wolf genes may have introgressed into the eastern coyote population by a study on the taxonomic origin of red wolves (*Canis rufus*) (Wilson et al. 2000). These authors identified a group of mtDNA, control region, sequences that were specific to red wolves and eastern Canadian wolves, and that are not found in the gray wolf. These genetic sequences also represent an additional marker that can be used to identify whether wolf genetic material has introgressed into coyote populations. Wilson (unpublished data) using the new genetic marker, and coyote samples from New York and New Brunswick, reported that these coyotes had hybridized with the eastern Canadian wolf (*C. lycaon*) resulting in a *C. latrans* x *lycaon* form.

Genetic evidence from Wilson et al. (2000) supports a close evolutionary history between the eastern Canadian wolf (presently *C. l. lycaon*) and the red wolf (*C. rufus*) that is independent of the gray wolf. Under this model, eastern wolves evolved in North America and shared a common ancestor with coyotes 150,000-300,000 years ago, with both *C. lycaon*/*C. rufus* and *C. latrans* being 1-2 million years divergent from the gray wolf (*C. lupus*). Gray wolves, on the other hand, are thought to have originated in the Old World and emigrated to the New World via the Bering land bridge approximately 300,000 years ago (Nowak 1979). Eastern wolves (*C. lycaon and C. rufus*) appear to readily hybridize with coyotes. Contrastingly, the absence of coyote DNA in western gray wolf populations that occur sympatrically with coyotes (e.g., Kenai, Alaska; Thurber and Peterson 1991) argues against the ability of gray wolves to hybridize with coyotes (Wilson et al. 2000, 2003). Based on the existing genetic evidence, Wilson et al (2000) and subsequent studies (Wilson et al. 2003, Grewal et al. submitted) suggests that the eastern Canadian wolf retain its original species designation, *C. lycaon* (Brewster and Fritts 1995). We use the terminology "eastern Canadian wolf" based on our frame of reference, but these wolves likely represent the once larger distribution of the eastern Canadian wolf that occupied the eastern portion of North America (Brewster and Fritts 1995). We also use the scientific nomenclature of *Canis lycaon* to denote the eastern Canadian wolf.

For this study, we sought to test the hypothesis that wolf genes have not introgressed into the eastern coyote population by examining the genetic profiles of three populations of eastern coyotes and comparing those profiles to populations of wolves and western coyotes. In addition, we wanted to collect information that could be used to address the issue of coyote/wolf hybridization as it applies to the feasibility of wolf recovery in the Northeast (e.g., Fascione et al. 2000). Wolf recovery in the Northeast has particular relevance to Maine, given that the state contains the most suitable habitat for wolves in the northeastern U.S. (Harrison and Chapin 1998, Mladenoff and Sickley 1998). Central to the issue of wolf recovery is the question of which species of wolf is the most appropriate to recover in the Northeast. Unfortunately, only two wolf specimens from the Northeast have been found and are available for taxonomic investigations

(Wilson et al. 2003). Therefore, we attempted to locate additional museum specimens of northeastern wolves for genetic analysis and classification.

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Materials & Methods

Samples and DNA Extraction

We analyzed tissue samples from populations of eastern Canadian wolves, eastern coyotes, western coyotes, and wolves from Quebec, and compared these animals to coyotes from Maine (n=100). In addition to samples obtained from Maine coyotes, eastern coyotes from Adirondack State Park, New York (n=66) and from the periphery of Kouchibouguac National Park, New Brunswick (n=20) were also analyzed. Western coyotes were represented by samples from Texas (n=24), Ohio (n=15), and North Carolina (n=22). Coyotes from Ohio and North Carolina were previously characterized as being genetically representative of western coyotes (Wilson, unpublished data). Wolves from Algonquin Provincial Park, Ontario (n=49) represented eastern Canadian wolves. Wolf–like canids were obtained from Quebec, specifically from the Laurentides (n=39), La Maurice National Park (n=10), and areas near the St. Lawrence River (at least one animal was taken south of the St. Lawrence River) (n=7). DNA was extracted following a modified Qiagen$_{TM}$ extraction protocol using the lysis buffer described in Guglich et al. (1994) from frozen tissue samples (liver, heart, kidney, or muscle).

Maine sample collection

Tissue samples and morphological measurements were obtained from 100 coyotes purchased from 13 snarers and hunters participating in a state sponsored animal damage control program from December 2000 to February 2001. Participants were instructed to turn in all coyotes, up to their prearranged limit, and not to select which coyotes to submit for the study. Coyotes were collected from 21 townships in Maine, primarily in the northern half of the state. Coyote dispersal patterns (Harrison 1992), and a genetic study on 45 Maine coyotes (Roger Denome, Stonehill College, unpublished report) indicated that coyotes from different areas of the state frequently mixed and that there was considerable gene flow among Maine coyotes. Therefore, although the majority of the coyotes used in this study were from the northern half of the state, the samples collected are believed to be representative of the general population of Maine coyotes.

Morphological Measurements

A single observer recorded skull (Nowak 1995) and body measurements for coyotes collected from Maine, and photographed each coyote from six angles (Table 1). Skulls were boiled, cleaned, and dried to a constant weight (60° C in a convection air oven) prior to taking measurements. Coyotes were aged by x-raying a lower canine and examining the pulp cavity. Coyotes having an open root canal or large pulp cavity were classified as juveniles, whereas yearlings (1-2 years) and adults (>2 years) had closed root canals and narrow pulp cavities (Linhart and Knowlton 1967). Study skins were collected systematically from every seventh coyote and from any unusual specimens.

Microsatellite Analysis

Eight microsatellite loci (Ostrander et al. 1993, Roy et al. 1994, 1996) were analyzed as described in Wilson et al. (2000).

Population Genetic Structure

Nei's unbiased genetic distance (Nei 1978) was calculated using the program PHYLIP (Felsenstein 1993). Neighbor-joining trees for each genetic distance were generated using the program NEIGHBOR in the computer package PHYLIP (Felsenstein 1993). Population genetic structure was estimated using the Weir and Cockerham (1984) estimate of F_{ST} using the software program ARLEQUIN (Schneider et al. 2000).

Bayesian Cluster Analysis

To determine the taxonomic nature of eastern coyotes from Maine, these genotypes were pooled in a set of additional *Canis* samples representative of eastern Canadian wolves (Algonquin Provincial Park, Ontario), western coyotes (Texas, Ohio, and North Carolina), eastern coyotes (Adirondacks, New York, and New Brunswick) and analyzed using the computer program STRUCTURE (Pritchard et al. 2000). STRUCTURE identifies multi-locus genotypes that are genetically similar without utilizing any known population affiliation, and provides the proportion of ancestry or the ancestry coefficient (q_i) in each cluster. The proportion of ancestry can be thought of as an index for individual animals that describes the average proportion of their genotype that is inferred to come from each cluster (e.g., a cluster may be made up of group of animals with a similar genetic makeup such as one might find in a population). The model assumes that populations are in Hardy-Weinberg equilibrium (HWE) and linkage equilibrium. Departure from equilibrium results in the identification of subpopulations to which individuals are assigned. Those individuals with mixed ancestry are assigned to more than one subpopulation or taxonomic cluster. We initially assessed the posterior probabilities of using four populations (MAXPOPS option = 4) assuming the presence of the following clusters: eastern Canadian wolves, western coyotes, eastern coyotes, and gray wolves. Following the assessment of the proportion of ancestry from gray wolves, we assessed the posterior probabilities of using three clusters (MAXPOPS option = 3) to generate the ancestry coefficients within the pooled *Canis* sample. For this assessment, we assumed the taxonomic groupings of eastern Canadian wolf, western coyote, and eastern coyote. We applied 1,000,000 iterations with a 30,000 burn-in period to determine the likelihood of the number K (the estimated number of subpopulations or genetic clusters) within the dataset (Pritchard et al. 2000).

Principal Component Analysis (PCA) of Genetic Profiles

A Principal Component Analysis of microsatellite profiles using PCAGEN (Goudet 1999) was applied to eastern Canadian wolves, eastern coyotes, gray wolves, western coyotes, and Maine coyotes to assess the overall relationship of individual canids from different regions.

Discriminant Function Analysis

Discriminant Function Analysis (DFA) was used to determine whether species could be distinguished based on their physical characteristics. This procedure determines which combination of physical characteristics (if any) best discriminates between groups of samples. Samples are then assigned to a particular group based upon the measurements of each sample's particular physical characteristics. If a large percentage of the samples are classified correctly (e.g., western coyote), one can conclude that group differences do exist and that the selected set of variables exhibits those differences. Alternatively, if a large percentage of samples fail to be correctly classified, then either the selected variables do not reflect any group differences or the groups must be homogeneous.

Because morphological measurements were collected during several independent investigations, the same morphological features were not measured on all animals. Therefore, only those morphological features, which were measured in all the investigations, could be used for analysis. Furthermore, juvenile animals were excluded from our analyses in order to remove any statistical bias resulting from underdevelopment.

Search for Historical Specimens

We attempted to locate wolf specimens (skeletal samples or hides) from New England by contacting museums, natural history societies, and taxonomists. Since earlier searches for wolf specimens had limited success in finding specimens from museums on the east coast, a special attempt was made to contact western museums and museums in Europe.

Results & Discussion

Population Genetic Structure

The genetic structure of animals from different geographies based on F_{ST} estimates (Weir and Cockerham 1984) indicated extensive gene flow between Maine canids and eastern coyotes from the Adirondacks, and New Brunswick (Table 2). Extensive gene flow among coyotes in Maine, New York, and New Brunswick is consistent with observations of lengthy dispersals of juvenile coyotes (e.g., 348 km, Harrison 1992), and movements of coyotes between Maine and New Brunswick (Jakubas, unpublished records).

However, coyotes from Maine, New Brunswick, and the Adirondacks showed less gene flow, i.e. higher levels of differentiation, when compared to western coyotes from Ohio, North Carolina, and Texas. Furthermore, the eastern coyotes of Maine, the Adirondacks, and New Brunswick showed significant differentiation to canid populations from Quebec, Algonquin Provincial Park, and Northwest Territories gray wolves. These patterns of differentiation were supported with estimates of Nei's (1978) genetic distance measure, which were calculated from the microsatellite allele frequencies. The topology of the neighbor-joining (NJ) tree of Nei's unbiased genetic distance (Fig. 2) paralleled the pairwise estimates of genetic differentiation, i.e. F_{ST}. The overall pattern of the NJ tree showed a very close relationship between Maine canids and eastern coyotes from the Adirondacks, and New Brunswick, which supports a common ancestry.

Bayesian Cluster Analysis

Nei's genetic distance and F_{ST} indicated that Maine coyotes are part of the same eastern coyote population in the Adirondacks and New Brunswick; however, these are indirect measures of genetic differentiation. The program STRUCTURE estimates the proportion of ancestry, and is a more direct estimate, with a higher resolution, than F_{ST} or Nei's genetic distance. The number of clusters or subpopulations (K) in the data set consisting of animals from Maine, Algonquin Provincial Park, Adirondack State Park, New Brunswick, Ohio, North Carolina, and Texas was set at three. The number of subpopulations was determined using the Bayesian algorithm to determine the ancestry of Maine canids in comparison to eastern Canadian wolves (*C. lycaon* x *latrans*), eastern coyotes of the Adirondacks and New Brunswick (*C. latrans* x *lycaon*), and western coyotes (*C. latrans*).

Consistent with Nei's genetic distance and the estimates of genetic differentiation, 93% (n = 100) of Maine's canids had an ancestry index similar to the typical eastern coyote (i.e., > 50% eastern coyote) (Fig.3). Five Maine canids had ancestries $\geq$ 30% eastern Canadian wolf, with one adult female having a genotype profile of 89% eastern Canadian wolf (Fig. 3). Surprisingly, only 4% of Maine coyotes had ancestries similar to western coyotes (i.e., >50% western coyote).

While not shown graphically, Maine eastern coyotes were compared to wolf-like canids from Laurentide Provincial Park, La Maurice National Park, and nearer the St. Lawrence River using the Bayesian cluster analysis. This analysis focused on determining whether Maine and Quebec canid populations were distinct, rather than on the taxonomy of individual animals. The population structure of Maine Coyotes appeared to be distinct (no ancestry detected) from the Quebec cluster, which was consistent with the F_{ST} and Nei's genetic distance estimates. Currently, there may be little opportunity for Maine coyotes to mix with canids north of the St. Lawrence River because of significant physical barriers (St. Lawrence River, urban areas, roads, and agricultural land) and trapping pressure south of the St. Lawrence River (Harrison and Chapin 1998, Wydeven et al. 1998).

Principal Component Analysis (PCA) of Genetic Profiles

The overall patterns of the PCA indicate general clustering of canids from specific regions (e.g., Adirondacks, New Brunswick) or taxa (e.g. eastern Canadian wolf, western coyotes; Fig. 4a). Gray wolves from NWT do not overlap eastern Canadian wolves or western coyotes. However, there is some overlap between eastern Canadian wolves and western coyotes, which is consistent with hybridization within the Canadian population of wolves in Algonquin Park, Ontario (Wilson et al. 2000). If eastern coyotes from the Adirondacks and New Brunswick are superimposed onto the PCA, more overlap is evident with eastern Canadian wolves and western coyotes. This supports a mixed hybrid ancestry for eastern coyotes from these regions. A number of eastern coyote samples group apart from the parental species, which is consistent with an eastward expansion following an initial hybridization event (likely in Ontario and southern Quebec). This pattern suggests these animals, despite having a hybrid origin, have diverged from one or both parental species. Superimposing Maine coyotes (Fig. 4b) onto the PCA supports the STRUCTURE results, in that these canids are consistent with eastern coyotes representing a *C. latrans* x *lycaon* mixture.

Morphological Measurements – Maine

Of the 107 coyotes collected for genetic and morphological measurements, 44.9% were females and 46.7% were >1 year of age. Only animals > 1 year of age were used for morphometric comparisons (Tables 3 and 4). Skull measurements were not made on seven coyotes because of badly fractured skulls. A comparison of photographs and study skins to the genetic profile of Maine coyotes (Appendix 1) did not indicate that coyotes with a high amount of wolf ancestry differed markedly in physical appearance from other coyotes. This analysis was confounded by the low number (n=5) of coyotes with wolf ancestries > 30% and the variability among those specimens.

The proportion of adult animals in the sample was higher than expected. Major (1983), who studied coyotes in Maine, reported 83% of the coyotes captured in conventional foothold traps were juveniles. Typically, juvenile animals are more vulnerable to trapping when they are dispersing (Harrison 1992). The period during which we collected snared animals fell after the major fall dispersal period (i.e., October and November) and during the first half of the second major dispersal period (i.e., February and March) (Harrison 1992). In addition, it may be more difficult for coyotes to learn how to avoid snares, as opposed to foothold traps, thus accounting for the higher ratio of adults to juveniles in our sample.

Discriminant Function Analysis

Eight morphometric measurements from the Quebec wolf population (n=94), the Quebec coyote population (n=19), and the Maine coyote population (n=49) were used to test for inter- and intra-population variation. These included weight (kg), hind foot length (cm), total length (i.e., zoological length) (cm), neck circumference (cm), shoulder height (cm), chest circumference (cm), lower canine length (cm), and upper canine length (cm), (Table 5). There was morphometrical variation among the sexes within each of the populations. Kruskal-Wallis non-parametric comparisons showed that males of the Quebec coyote population had a greater shoulder height and longer lower canine teeth than that of females (χ^2=3.14, p=0.007 and, χ^2=4.524 p=0.03). In the Maine coyote population, male coyotes had a greater total length than females (χ^2=11.18, p<0.001), larger neck circumference (χ^2=16.67, p<0.001), greater shoulder height (χ^2=10.47, p<0.001), and longer lower and upper canine teeth (χ^2=13.09, p<0.001;χ^2=20.58, p<0.001 respectively). When morphometric data was tested for variation among the three populations, all characters were significantly different for males (p<0.001), females (p<0.01), and total population (p<0.001).

To further analyze morphometrical variation among the three *Canis* populations, we implemented the use of a discriminant function analysis. Discriminant function analysis is used to determine which variables discriminate between two or more naturally occurring groups. Of the eight morphometrical variables entered into the analysis, six were deemed as valid predictors for population characterization predictors (α = 0.05). These included hind-foot length, total length, neck circumference, shoulder height, lower canine length, and upper canine length. Our analysis showed that by using these morphometrical variables 79.6% of the individuals were correctly assigned to their proper population. However, 27 individuals of the Quebec wolf population were classified as Quebec coyotes and 5 individuals were assigned to the Maine coyote population. One canid from Maine was classified as being morphometrically similar to Quebec coyotes. All of the Quebec coyotes were correctly classified as Quebec coyotes (Table 6).

These results suggest that hybridization may be occurring between the Quebec wolf and coyote populations. However, it is necessary to perform a more detailed analysis with more morphometrical variables. One drawback from this analysis was the dissimilarity of data sets for each population, where not all of the morphological measurements were collected for each population. These results are consistent with the genetic data suggesting little or no gene flow or connectivity exists between Quebec canids and Maine coyotes. Juvenile animals from a wider range of locations are presently being analyzed, but the variance in this age category may influence the accuracy of the analysis.

Search for Historical Specimens

Over 42 museums and numerous individuals were contacted to determine whether any wolf specimens from New England were in their collections (Appendix 2). No additional specimens were found other than those previously located at the Museum of Comparative Zoology at Harvard and the Adirondack Museum in Blue Mountain Lake, New York. Included in our list of museums is a search done by Ron Nowak, USFWS (Appendix 2). One problem we identified in locating specimens was that many museums did not have computerized inventories. Consequently, it was difficult for many museums to determine whether they had any specimens of interest to us without spending considerable staff time on the search, which they often could not provide.

Interpretation of Ancestry in Eastern Canids

We reject the hypothesis that wolf genes have not introgressed into the eastern coyote population. The genetic structure of coyote populations from Maine, New York, and New Brunswick were closely related based on measures of genetic distance (F_{ST} estimates, Nei's [1978] genetic distance measure). These coyote populations, in turn, shared similar genetic ancestries, which included hybridization with eastern Canadian wolves (Bayesian cluster analysis). Finally, these eastern coyote populations showed a degree of genetic overlap with eastern Canadian wolves (Principle Component Analysis) that was consistent with a *C. latrans* x *lycaon* mixture. Our morphological comparisons suggest that Quebec wolf and coyote populations are hybridizing. However, a more detailed morphological analysis is necessary to be definitive.

The hybridization of eastern coyotes (*C. latrans x C. lycaon*) poses several challenges to the management of coyotes and the protection of wolves emigrating from Canada to the northeastern U. S. In Maine, coyotes are one of the most popular species for upland trappers to pursue (Jakubas 2003), and are also harvested by snarers working as animal control agents and hunters. In all these activities, the public must rely on the morphological characteristics of the animal to distinguish whether the animal is a coyote or a wolf. However, our study indicates that morphological characteristics may not always give a clear indication of the species of the animal. In addition, our study indicates there are limitations when using genetic profiles to differentiate eastern Canadian wolves from eastern coyotes, because both canids have composite hybrid genomes. For example, canids from Algonquin Park, Ontario were included in this study as representative eastern Canadian wolves. Four of these animals were among the smallest animals and had the following ancestries: 15.5 kg (34.2 lb) - 95.5% eastern coyote; 18.0 kg (39.6 lb) - 97.5% eastern Canadian wolf; 18.1 kg (39.9 lb) - 91.8% eastern Canadian wolf; and 14.0kg (30.9 lb) - 41.5% eastern Canadian wolf and 41.5% eastern coyote. In addition, the ancestry of one adult female from Maine was 89% eastern Canadian wolf, but it was one of the smallest animals sampled at 12.3 kg (27.0 lb). The presence of a small canid in Maine with a high wolf ancestry does not necessarily indicate that an eastern Canadian wolf immigrated into Maine. Eastern coyotes contain "neutral" alleles from two parental species (*C. latrans and C. lycaon*);

therefore, offspring of eastern coyotes may occasionally exhibit genotypes more similar to eastern Canadian wolves. For example, Tweed wolves from the Frontenac Axis (south of Algonquin Park) have ancestries similar to Algonquin wolves, and are difficult to distinguished from the Algonquin wolves or offspring of eastern coyotes that contain eastern Canadian wolf alleles. These examples illustrate the confusion that may result when trying to determine whether a canid is a wolf or coyote. Some of the challenges that wildlife agencies face include developing workable standards for identifying canids in the northeast, determining the degree of protection that can be given to wolves in a hybrid zone, and devising management plans that will provide that protection.

The results from this study raise the question, why isn't an animal's genetic ancestry better reflected in its appearance? The relationship between the genotype assessment and phenotype of the animal may not be directly correlated for a number of reasons. Microsatellites are effectively neutral DNA markers, and therefore, do not contribute to the morphology of individual animals. Secondly, the introgression of genetic material through hybridization may be historic, such that continual inter-breeding with one of the parental species, in this case the eastern Canadian wolf is limited. Combinations of alleles resulting from breeding within a hybrid gene pool can generate profiles similar to one of the parental species by chance. The morphology of the canids will further be modified by responses to selection, overall health of the animal, diet, and development -- factors independent of the neutral genotypes observed with microsatellites.

Additional morphological evidence for overlap between eastern Canadian wolves and eastern coyotes comes from a study by David Pennock (Fort Hays State University, Kansas; personal communication). In this study, several key measurements (greatest length of skull, incisor width, temporal fossa, and zygomatic width) showed overlap between eastern coyotes in southern Ontario (similar to Maine animals) and eastern Canadian wolves in Algonquin Park, Ontario, although greatest skull length was more distinguishing than the other measurements. Boreal wolves, corresponding to *C. lupus* x *lycaon* animals, were distinguished from eastern coyotes. Thus, skull-length measurements may provide a means to distinguish the larger Quebec Laurentide-type wolves, moving south into Maine from Maine coyotes, when a carcass is available to examine, but would be of little use in the field identification of live animals.

In general, the approaches used in this study provide an assessment of the ancestry between eastern Canadian wolves and coyotes and provide evidence supporting their hybrid ancestry. These interpretations are based on defining the overall population of eastern coyotes in Maine. The approach provides limited utility for determining the species of an individual canid because of overlap in genotypes and phenotypes within the larger hybrid gene pool of eastern Canadian wolves and eastern coyotes. The ability to distinguish eastern coyotes from other wolves, such as gray wolves, and gray wolf x eastern Canadian wolves is feasible as observed with the nearest gray wolves (gray x eastern Canadian wolf) in Laurentides Reserve, Quebec (Fig. 4a) (also see Mech and Federoff 2002, Grewal et al. submitted). These gray wolf-like animals are morphologically more distinct than eastern Canadian wolves found in Algonquin Park, Ontario and eastern coyotes in Maine. The ability to distinguish eastern coyotes from expanding western coyotes that have not hybridized may be feasible if an increased number of loci were used.

Conclusion

The majority of Maine canids are eastern coyotes with a hybrid origin and a designation of *C. latrans* x *lycaon.* The major difference between eastern coyotes and their western counterparts is their larger size (Richens and Hugie 1974, Lawrence and Bossart 1975). Similar to western coyotes, the eastern coyote hybrid is a highly adaptable animal and inhabits a range of habitats from forested regions in the Adirondacks, New Brunswick, and Maine to more agriculturally developed areas (Parker 1995).

The view that natural selection acted alone on eastern coyotes, in the absence of wolf gene introgression (Schmitz and Lavigne 1987 and Thurber and Peterson 1991), to produce an animal with a larger body size is not supported by this study. However, an alternative hypothesis, that natural selection acted on introgressed genetic material (*C. latrans x lycaon*) to produce these characteristics cannot be rejected. The introgression of eastern Canadian wolf genes into eastwardly expanding coyotes could have provided a composite genome that facilitated selection of animals with a larger body size and that may be more adept at preying on deer than smaller western coyotes. Any interpretations of genetic-environmental interactions would be premature based on the neutral nature of microsatellites and the number of loci employed in this study. However, the adaptable eastern coyote is likely an important model to examine the interaction between genetic profiles (wolf-like vs. coyote-like) and morphological adaptations to different prey and habitat.

The functional role and adaptive potential of eastern coyotes should be factored into conservation considerations surrounding wolf recovery in the east. The question of whether it is preferable to recover a "pure" species or maintain the functional role and adaptive potential of a species through hybridization has arisen before during recovery efforts for the Florida panther (*Felis concolor coryi*) (O'Brien and Mayr 1991, Hedrick 1995). In this case, the long-term viability of the species and the retention of adaptive alleles present in the Florida panther was thought to be best served by allowing hybridization to occur with cougar from Texas (Hedrick 1995). Recent discussions on the effects of hybridization on evolutionary processes and on conservation efforts for endangered species recognize the importance of preserving ecological function, adaptive alleles, and the role of hybridization events in the evolution of a species (Crandall et al. 2000, Allendorf et al. 2001, Reiseberg et al. 2003). In the case of the hybrid eastern coyotes, the adaptive potential is important as it contains the genetic material of the parental species, e.g. *C. lycaon* x *C. latrans* -- genetic material that may have been lost without the timely expansion of the sister-species into the reduced range of the eastern Canadian wolf and red wolf. The presence of coyote genes in a wolf x coyote hybrid raises the possibility that the most well adapted wolf-like canid for a human altered landscape (i.e., able to persist) may be the introgressed form.

At present, the coyote/wolf hybrids inhabiting the Adirondacks, New Brunswick, and Maine serve a functional role as top end predators - preying on white-tailed deer (Litvaitis and Harrison 1989, Brundige 1993, Parker 1995) but not moose. This is similar to the eastern Canadian wolf, which predominantly preys on deer rather than moose (i.e. < 10% of its diet; Forbes and Theberge 1996). Currently, the closest wolf population to

Maine is in the Laurentides Reserve, Quebec (Fig. 1). These canids appear to be hybrids of the gray wolf and eastern Canadian wolves (Fig. 2), and are adept at preying on moose (Jolicoeur 1998). In all likelihood, southern Quebec (e.g., the Laurentides) and southern Ontario (e.g., Pukaskwa National Park, Grewal et al. submitted) had gray wolves prior to European settlement, and there is no reason to suspect that gray wolves from these areas couldn't have immigrated into New England during the era when moose and caribou were prevalent in northern Maine. However, to date, the only historic specimens of wolves from the northeastern U.S. (n = 2) have been identified as *C. lycaon* (Wilson et al. 2003).

Questions, such as, what additional benefits would eastern Canadian wolves or gray wolves bring to ecosystems in the Adirondacks and New England States, need to be addressed. A comparison of the ecological differences between eastern Canadian wolves and eastern coyotes should be undertaken to determine what differences exist between the two predators. If the reintroduction of the eastern Canadian wolf is intrinsically important, because it historically existed in the northeastern U.S., then the feasibility of maintaining such a population of *C. lycaon* in proximity to populations of eastern coyotes must be addressed. The issue of coyote/wolf hybridization is not abstract, as it has been identified as the most significant issue facing the success of the red wolf re-introduction program (USFWS 1999). Consideration of the Algonquin model (Wilson et al. 2000) and factors influencing the barriers to gene flow among canid populations in the Northeast are important in assessing management strategies for maintaining eastern wolves (*C. lycaon* and *C. rufus*) within eastern U.S. ecosystems.

Literature Cited

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution,* **16**, 613-622.

Bowen WD (1981) Variation in coyote social organization: the influence of prey size. *Canadian Journal of Zoology* **59**, 639-652.

Brewster WG, Fritts SH (1995) Taxonomy and genetics of the gray wolves in western North America. In: *Ecology and conservation of wolves in a changing world: proceedings of the second North American symposium of wolves* (eds. Carbyn LN, Fritts SH, and Seip DR), pp. 353-373. Canadian Circumpolar Institute, University of Alberta, Edmonton, Canada.

Brundige GC (1993) Predation ecology of the eastern coyote, *Canis latrans* var., in the Adirondacks, New York. Ph.D. Dissertation, State University of New York, Syracuse.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution,* ***15****, 290-295.*

Fascione N, Byers O, and Seal US (*eds.*) (2000) Wolves in the Northeast? *A workshop to exchange views. Final report. IUCN/SSC Conservation Breeding Specialist Group*: Apple Valley, MN. 133pp.

Felsenstein J (1993) *PHYLIP - phylogenetic inference package, version 3.5c.* University of Washington, Seattle.

Forbes G J, Theberge J B (1996) Response by wolves to prey variation in Central Ontario. Canadian Journal of Zoology, **74**, 1511-1520.

Gaskin P (1975) A multivariate analysis of skull characteristics of New York coyotes. Trans. *Eastern Coyote Workshop, NE Fish Wildlife Conference, New Haven, Conn. 5-12.*

Gese E M, Grothe S (1995) Analysis of coyote predation on deer and elk during winter in Yellowstone National Park, Wyoming. *American Midland Naturalist,* **133**, 36-43.

Grewal S K, Wilson P J, Granacki A D, Heal J N, Lawford I, Hillis T L, Mallory F F, Sears H, Theberge M T, Theberge J B, Voigt D R, Burrows F, White B N (submitted) Characterization of wolves across Ontario using mitochondrial and microsatellite DNA profiles. *Conservation Genetics.*

Goudet, J (1999) PCA-GEN for Windows, Version 1.2.

Guglich E A, Wilson P J, White B N (1994) Forensic application of repetitive DNA markers to the species identification of animal tissues. *Journal of Forensic Science,* **39**, 353-361.

Harrison DJ (1992) Dispersal characteristics of juvenile coyotes in Maine. *Journal of Wildlife Management,* **56**, 128-138.

Harrison DJ, Chapin TG (1998) Extent and connectivity of habitat for wolves in eastern North America. *Wildlife Society Bulletin,* **26**, 767-775.

Hedrick PW (1995) Gene flow and genetic restoration: the Florida panther as a case study. *Conservation Biology*, **9**, 996-1007.

Hilton H (1978) Systematics and ecology of the eastern coyote. In: *Coyotes biology, behavior, and management.* (ed. Bekoff, M) pp. 209-228. Academic Press, San Diego.

Jakubas W J (2003) Trapper information 2002-2003. Maine Department of Inland Fisheries and Wildlife. Augusta, pp. 32.

Jolicoeur H (1998) Le loup du massif du lac Jaques-Cartier. Ministère de l'Environnement et de la Faune, Québec, 132 pp.

Lawrence B, Bossert WH (1975) Relationships of North American *Canis* shown by multiple character analysis of selected populations. In: *The wild canids*. (ed. Fox, MW) pp. 73-86. Van Nostrand Rienhold, New York.

Lehman N, Eisenhauer A, Hansen K, Mech D L, Peterson R O, Gogan P J P, Wayne R K (1991) Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. *Evolution*, **45**, 104-119.

Linhart S B, Knowlton, F F (1967) Determining age of coyotes by tooth cementum layers. *Journal of Wildlife Management,* **31,** 362-365.

Litvaitis J A, Harrision D J (1989) Bobcat-coyote niche relationships during a period of coyote population increase. *Canadian Journal of Zoology*, **67,** 1180-1188.

Major JT (1983) Ecology and interspecific relationships of coyotes, bobcats, and red foxes in western Maine. Ph.D. Thesis, University of Maine, Orono. 64pp.

Mech D L, Federoff N E (2002) α_1 – Antitrypsin polymorphism and systematics of eastern North American wolves. *Canadian Journal of Zoology,* **80**, 961-963.

Messier F, Barrette C, Huot J (1986) Coyote predation on a white-tailed deer population in southern Quebec. *Canadian Journal of Zoology,* **64**, 1134-1136.

Mladenoff D J, Sickley T A (1998) Assessing potential gray wolf restoration in the northeastern United States: a spatial prediction o favorable habitat and population levels. *Journal of Wildlife Management*, **62**, 1-10.

Moore G C, Parker G R (1992) Colonization by the eastern coyote (*Canis latrans*). In: *Ecology and management of the eastern coyote*. (ed. Boer AH) pp. 23-37. Wildlife Research Unit, University of New Brunswick, Fredericton, New Brunswick.

Nei M (1978) Estimation of the Average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583-590.

Nowak R M (1979) *North American Quartenary Canis*. University of Kansas Museum of Natural History Monograph, no. 6.

Nowak R M (1995) Another look at wolf taxonomy. In: *Ecology and conservation of wolves in a changing world.* (eds. Carbyn LN, Fritts SH, and Seip DR), pp. 375-398. Canadian Circumpolar Institute, University of Alberta, Edmonton, Canada.

O'brien S J, Mayr E (1991) Bureaucratic mischief: recognizing endangered species and subspecies. *Science*, **251**, 1187-1188.

Ostrander E A, Sprague G F, Rine J (1993) Identification and Characterization of dinucleotide repeat (CA)n markers for genetic mapping in dog. *Genomics,* **16**, 207-213.

Parker G R (1995) *Eastern coyote the story of its success*. Nimbus Publishing, Halifax, N.S. 254pp.

Pilgrim K L, Boyd D K, and Forbes S H (1998) Testing for wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. *Journal of Wildlife Management,* **62**, 683-689.

Pritchard J K, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Richens V B, Hugie R D (1974) Distribution, taxonomic status, and characteristics of coyotes in Maine. *Journal of Wildlife Management,* **38**, 447-454.

Rieseberg L H, Raymond O, Rosenthal D M, Lai Z, Livingstone K, Nakazato T, Durphy J L, Schwarzbach A E, Donovan L A, and Lexer C (2003) Major ecological transitions in wild sunflowers facilitated by hybridization.

Sciencexpress, DOI:10.1126/science.1086949, Published online August 7, 2003.

Roy M S, Geffen E, Smith D, Ostrander EA, Wayne R K (1994) Pattern of differentiation and hybridization in North American wolf like canids revealed by analysis of microsatellite loci. *Molecular Biology and Evolution,* **11**, 553-570.

Roy M S, Geffen E, Smith, D Wayne, R K (1996) Molecular genetics of pre-1940's red wolves. *Conservation Biology,* **10**, 1413-1424.

Schmitz O J, Lavigne D M (1987) Factors affecting body size in sympatric Ontario *Canis*. *Journal of Mammalogy,* **68**, 92-99.

Schneider S, Roessli D, Excoffier L (2000) *ARELQUIN 2.00 A software for population genetic analysis*. Genetics and Biometry Laboratory, University of Geneva.

Silver H, Silver W T (1969) Growth and behavior of the coyote-like canid of northern New England with observations of canid hybrids. *Wildlife Monographs No.17.*

Thurber J M, Peterson R O (1991) Changes in body size associated with range expansion in the coyote (*Canis latrans*). *Journal of Mammalogy,* **72**, 750-755.

USFWS (1999) Population and Habitat Viability Assessment (PHVA) for the Red Wolf (*Canis rufus*), Briefing Book, Virginia Beach, Virginia, April 13-16, 1999.

Wayne R K, Lehman N, Allard M W, Honneycutt R L (1992) Mitochondrial DNA variability of the gray Wolf: Genetic consequences of population decline and habitat fragmentation. *Conservation Biology,* **6**, 559-567.

Wayne R K, Lehman N (1992) Mitochondrial DNA analysis of the eastern coyote; origins and hybridization. In: *Ecology and Management of the Eastern coyote*. (ed. Boer AH) pp. 9-22. Wildlife Research Unit, University of New Brunswick, Fredericton, New Brunswick.

Weir B S, Cockerham C C (1984) Estimating F-statistics for the analysis of population structure. *Evolution,* **38**, 1358-1370.

Wilson P J, Grewal S K, Lawford I D, Heal J N M, Granacki A G, Pennock D, Theberge J B, Theberge M T, Voigt D R, Waddell W, Chambers R E, Paquet P C, Goulet G, Cluff D, White B N. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. *Canadian Journal of Zoology,* **78**, 2156-2166.

Wilson P J, Grewal T, McFadden T, Chambers R C, and White B N (2003) Mitochondrial DNA extracted from eastern North American wolves killed in the 1800s is not gray wolf origin. *Canadian Journal of Zoology* **81**, 936-940.

Wydeven A P, Fuller T K, Weber W, and MacDonald K (1998) The potential for wolf recovery in the northeastern United States via dispersal from southeastern Canada. *Wildlife Society Bulletin* **26**, 776-784.

Table 1. Morphological measurements and photographs taken on Maine coyotes. Skull measurements were taken as according to Nowak (1995).

Body Measurements	Skull Measurements	Photographs
Weight	Greatest length	Lateral full body
Head length	Zygomatic width	Lateral head
Zygomatic arch	Alveolar length	Dorsal full body
Ear length	Greatest width of upper cheek teeth	Dorsal head
Neck circumference	Palatal width first premolar	Ventral full body
Chest girth	Frontal shield width	Ventral head
Shoulder height	Tooth row to Orbit Height	
Total length	Depth of Jugal	
Zoological length	Upper Carnassial Crown Length	
Tail length	Upper Molar Crown Width	
Upper canine length and width		
Distance between upper canines		
Lower canine length and width		
Distance between lower canines		
Nose pad width		
Front right heel pad length and width		
Front right foot total length		
Hind foot total length		
2nd Thoracic Nipple width and height		
Vulva width and length		

Table 2. F_{ST} values for pairwise comparisons of *Canis* from different regions. Lower values indicate higher levels of gene flow and less differentiation.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. Maine	0.00000							
2. LaMaurice	0.07575	0.00000						
3. St. Lawrence	0.05299	-0.00635	0.00000					
4. Laurentide	0.12690	0.02924	0.01344	0.00000				
5. Adirondack	0.01135	0.07365	0.06671	0.14274	0.00000			
6. New Brunswick	0.01231	0.09730	0.06247	0.12998	0.03325	0.00000		
7. Algonquin	0.09705	0.05175	0.05932	0.07267	0.10560	0.09443	0.00000	
8. Western Coyote	0.07114	0.05753	0.06107	0.12440	0.07267	0.10387	0.11221	0.00000

Table 3. Body characteristics of adult Maine coyotes presented as mean values ± standard error (SE); with sample size (n). All measurements are given in millimeters unless otherwise noted.

	Female		Male	
Measurement	Mean ± SE	n	Mean ± SE	n
Weight (kg)	14.2 ± 0.3	19	16.6 ± 0.4	28
Zoological length	1199 ± 10	21	1248 ± 9	28
Tail length	332 ± 5	21	346 ± 5	28
Shoulder height	585 ± 7	21	619 ± 5	28
Chest girth	494 ± 7	21	542 ± 9	28
Neck circumference	305 ± 4	21	335 ± 5	28
Head length	204 ± 2	21	214 ± 2	28
Zygomatic arch	103 ± 2	21	109 ± 2	28
Ear length	105 ± 1	21	106 ± 1	28
Nose pad width	24 ± 0	21	26 ± 0	28
Upper canine length	18.4 ± 0.4	21	21.3 ± 0.3	28
Upper canine width	9.0 ± 0.1	21	9.7 ± 0.1	28
Distance between upper canine tips	34.3 ± 0.3	21	36.0 ± 0.7	27
Lower canine length	16.8 ± 0.3	21	18.3 ± 0.2	27
Lower canine width	8.7 ± 0.2	21	9.4 ± 01	27
Distance between lower canine tips	30.4 ± 0.4	21	32.3 ± 0.3	27
Front right heel pad length	29 ± 1	21	31 ± 0	28
Front right heel pad width	27 ± 1	21	30 ± 1	28
Front right foot total length	77 ± 1	21	80 ± 1	28
Hind foot total length	198 ± 3	20	206 ± 2	28
2nd Thoracic Nipple width	3.4 ± 0.3	12		
2nd Thoracic Nipple height	5.0 ± 0.3	12		
Vulva width	3.5 ± 0.2	21		
Vulva length	11.3 ± 0.6	21		

Table 4. Skull characteristics of adult Maine coyotes presented as mean values ± standard error (SE); with sample size (n). All measurements are given in millimeters.

	Female		Male	
Measurement	Mean ± SE	n	Mean ± SE	n
Greatest length	192.8 ± 1.4	21	203.0 ± 1.4	27
Zygomatic width	100.5 ± 0.8	21	107.8 ± 0.5	28
Alveolar length maxillary toothrow	66.5 ± 0.9	21	70.2 ± 0.6	27
Maximum width upper cheek teeth	56.9 ± 0.5	21	60.8 ± 0.4	26
Palatal width	21.1 ± 0.2	21	22.3 ± 0.3	27
Frontal shield width	47.1 ± 0.7	21	50.1 ± 0.7	28
Height from toothrow to orbit	27.0 ± 0.3	21	28.8 ± 0.4	28
Jugal depth	12.6 ± 0.2	21	14.0 ± 0.3	28
Upper carnassial crown length	17.5 ± 0.2	21	19.3 ± 0.2	28
Upper M2 crown width	11.5 ± 0.1	21	12.2 ± 0.1	28

Table 5. Morphometrical data recorded from Quebec wolf, Quebec coyote, and Maine coyote populations.

	T Sex	Weight (kg)	Hind Foot Length (cm)	Total length (cm)	Neck Circum. (cm)	Shoulder Height (cm)	Chest Circum. (cm)	Lower Canine Length (cm)	Upper Canine Length (cm)
Quebec Wolf	**Male**	28.0±0.8 (13-58)[a]	25.6±0.3 (21-30)	163.3±2.3 (129-224)	38.0±0.8 (30-50)	75.5±1.0 (66-89)	67.7±0.9 (55-92)	2.2±0.08 (1.6-2.6)	2.4±0.08 (1.1-2.9)
	Female	27.4±0.8 (14-45)	23.7±0.2 (22-30)	158.7±2.4 (137-182)	36.7±0.9 (27-47)	76.2±3.8 (52-114)	66.6±0.7 (52-79)	2.3±0.03 (1.9-3.2)	2.5±0.03 (2.0-3.3)
	Total	27.8±0.8 (13-58)	25.7±0.2 (21-30)	162.7±1.4 (129-224)	37.9±0.5 (27-50)	75.3±0.7 (66-114)	67.2±0.8 (52-92)	2.4±0.03 (1.6-3.2)	2.4±0.04 (1.1-3.3)
Quebec Coyote	**Male**	13.6±0.6 (11-21)	20.9±0.3 (19-23)	137.4 ±1.1 (127-147)	29.8±0.7 (25-36)	60.5±0.4 (58-66)	52.8±0.6 (47-70)	1.9±0.08 (1.4-2.8)	1.9±0.09 (1.0-2.3)
	Female	12.5±0.1 (12-14)	14.7±1.0 (11-22)	130.3±0.5 (126-136)	28.3±0.1 (27-30)	54.2±0.3 (52-58)	48.3±0.1 (45-50)	1.7±0.03 (0.9-1.9)	1.5±0.05 (0.8-1.8)
	Total	13.3±0.5 (11-21)	19.1±0.6 (11-23)	136.2±0.6 (126-147)	29.7±0.3 (25-36)	64.0±0.3 (52-66)	51.5±0.5 (45-70)	1.8±0.04 (0.9-2.8)	1.8±0.04 (0.8-2.3)
Maine Coyote	**Male**	16.6±0.4 (15-21)	20.5±0.2 (19-23)	124.8±0.9 (114-134)	33.5±0.5 (25-36)	61.8±0.5 (57-70)	54.1±0.8 (45-63)	1.8±0.02 (1.2-2.2)	2.1±0.03 (1.9-2.4)
	Female	14.2±0.3 (12-18)	19.8±0.3 (17-22)	119.9±1.0 (111-127)	30.4±0.4 (27-35)	58.5±0.6 (51-62)	49.4±0.7 (45-55)	1.7±0.02 (1.4-1.8)	1.8±0.04 (1.4-2.0)
	Total	15.6±0.4 (12-21)	20.5±0.2 (17-23)	125.6±0.3 (111-134)	29.5±0.3 (25-36)	61.2±0.5 (51-70)	53.8±0.6 (45-63)	1.8±0.02 (1.2-2.2)	2.0±0.02 (1.4-2.4)

[a] measurements listed as mean ± standard error with range subtended by parenthesis.

Table 6. The number and percentage of animals from reference populations that were assigned to groups based on the morphological characteristics of the animal. Animals were assigned to groups following a discriminant function analysis on eight morphological variables, from which six variables (total length, hind foot length, shoulder height, neck circumference, lower canine length, and upper canine length) were identified as the best predictors. Overall, 79.6% of the animals were assigned to the correct group.

	Predicted Group Membership			
Reference Population	**Quebec Wolf**	**Quebec Coyote**	**Maine Coyote**	**Total**
Quebec Wolf (number of animals)	62	27	5	94
Quebec Coyote	0	19	0	19
Maine Coyote	0	1	48	49
Quebec Wolf (percentage of animals)	66.0	28.7	5.3	100
Quebec Coyote	0	100	0	100
Maine Coyote	0	2	98.0	100

Figure 1. Map of eastern North America showing regions of *Canis* sample collection. Circles indicate specific geographic designations and labels exclusively indicate the geographic resolution at the State level.

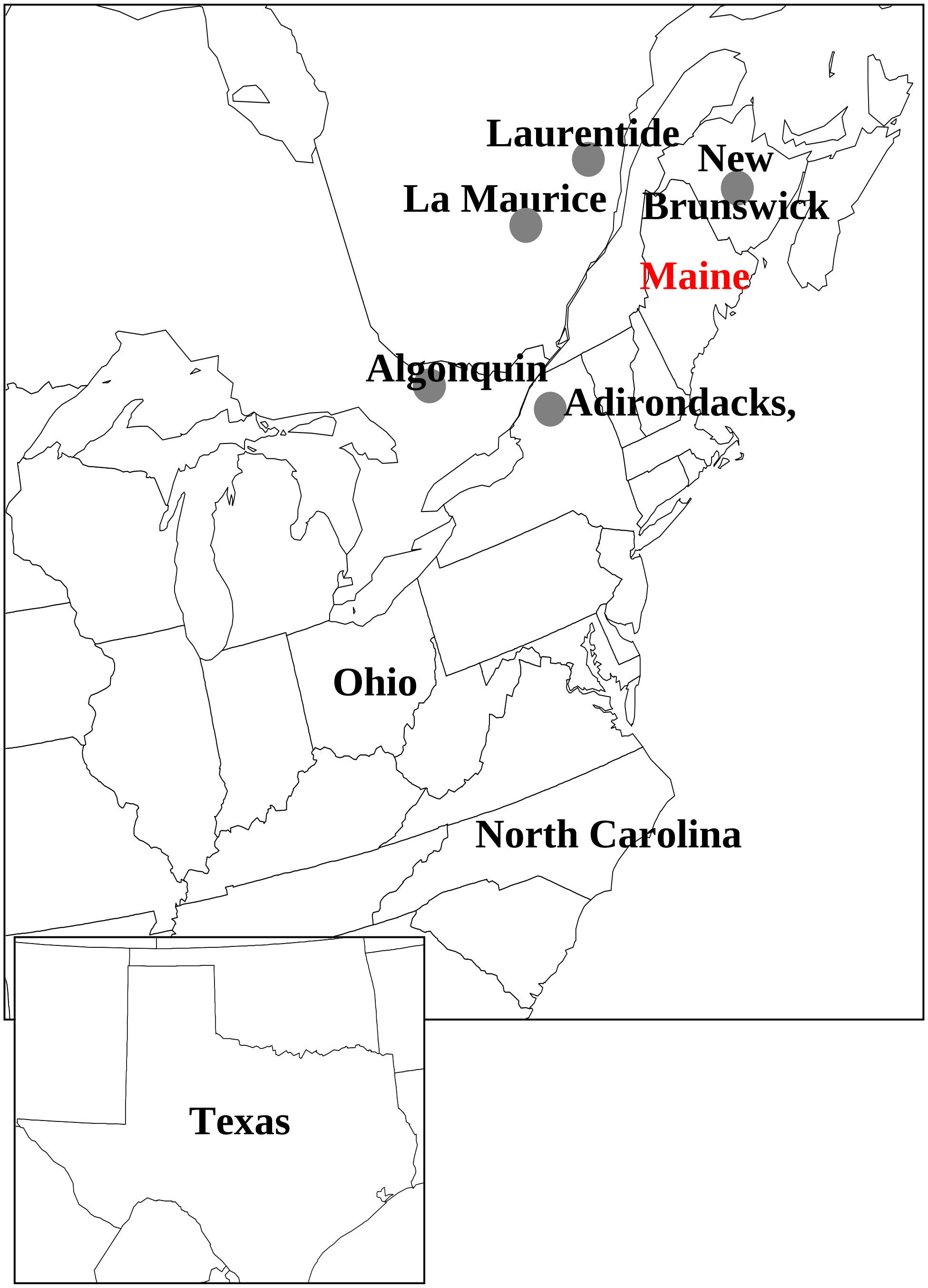


Figure 2. Unrooted neighbor-joining tree of Nei's unbiased genetic distance (Nei 1978) for *Canis* sample locations.

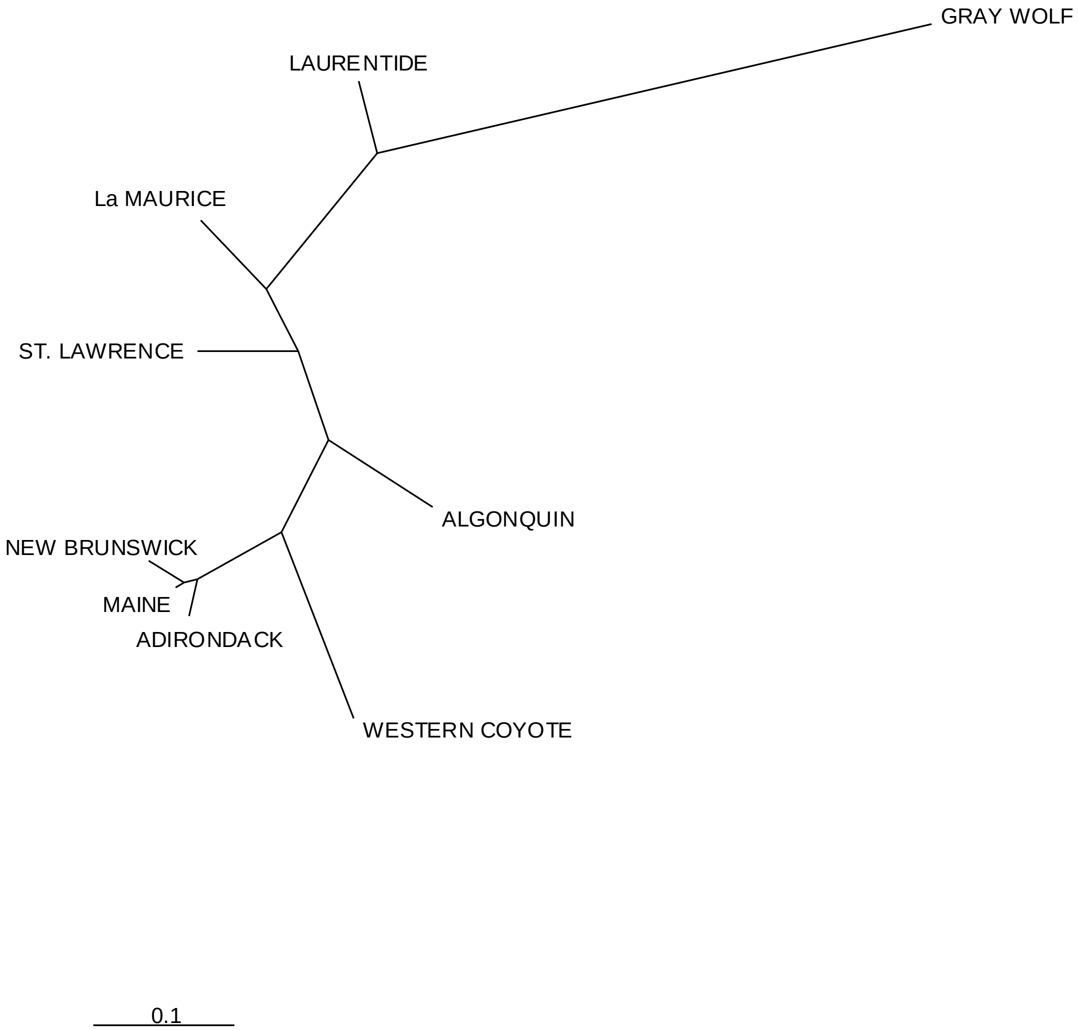


Figure 3. Bayesian clustering results of eastern *Canis* specimens showing three ancestry coefficients (q_i) in a ternary graph with the respective ancestries given by the distances to the three sides of the equilateral graph. Maine samples were compared to eastern coyote specimens from Adirondacks and New Brunswick and representative eastern Canadian wolves from Algonquin Provincial Park and western coyotes from North Carolina, Ohio and Texas. Cluster I is representative of animals having an eastern wolf (*C. lycaon*) ancestry, Cluster II is representative of animals having a western coyote ancestry (*C. latrans*), and Cluster III is representative of a *C. lycaon x C. latrans* hybrid.

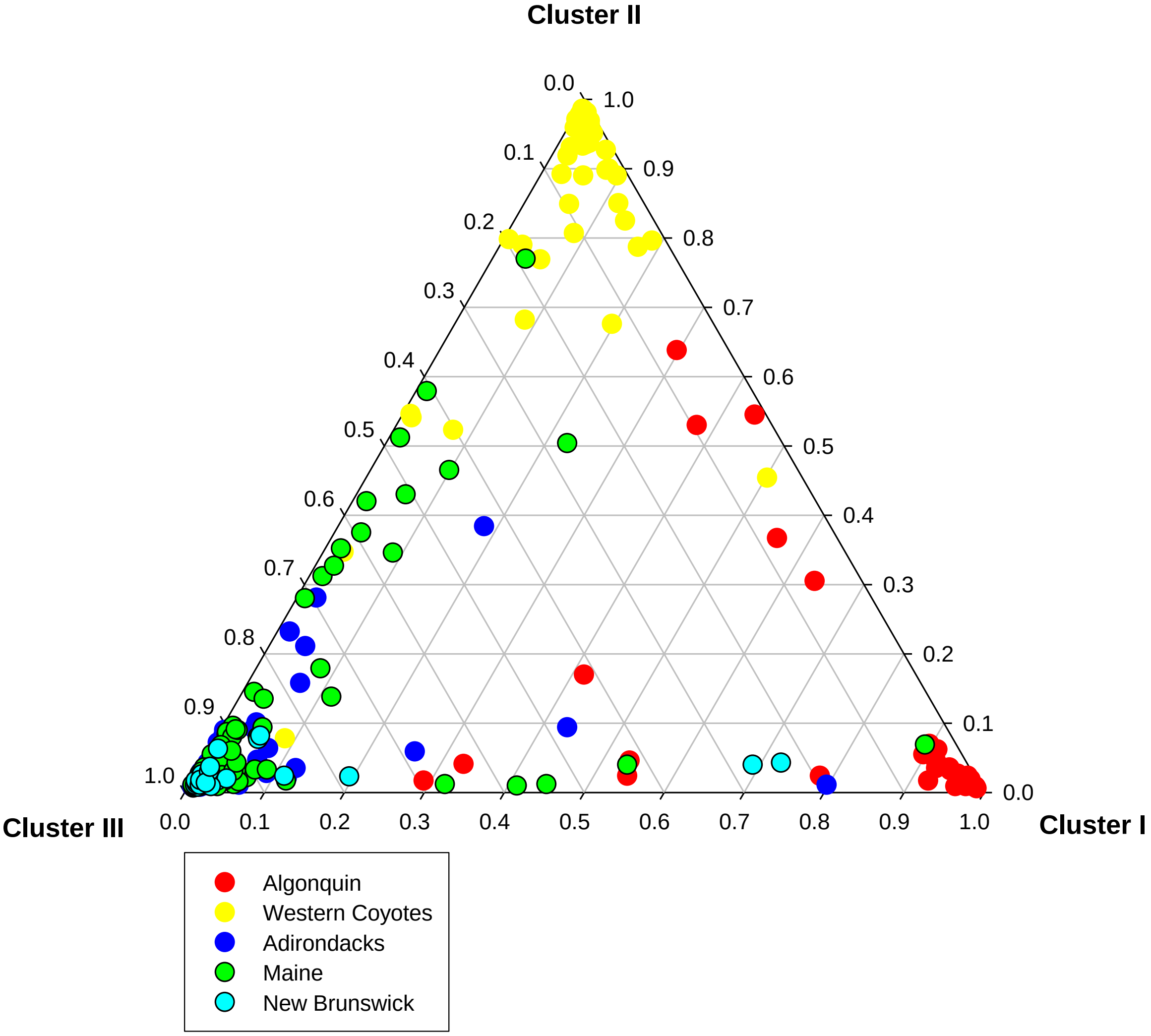


Figure 4a. Principal Component Analysis (PCA) of eastern North American canids using 8 microsatellite loci.

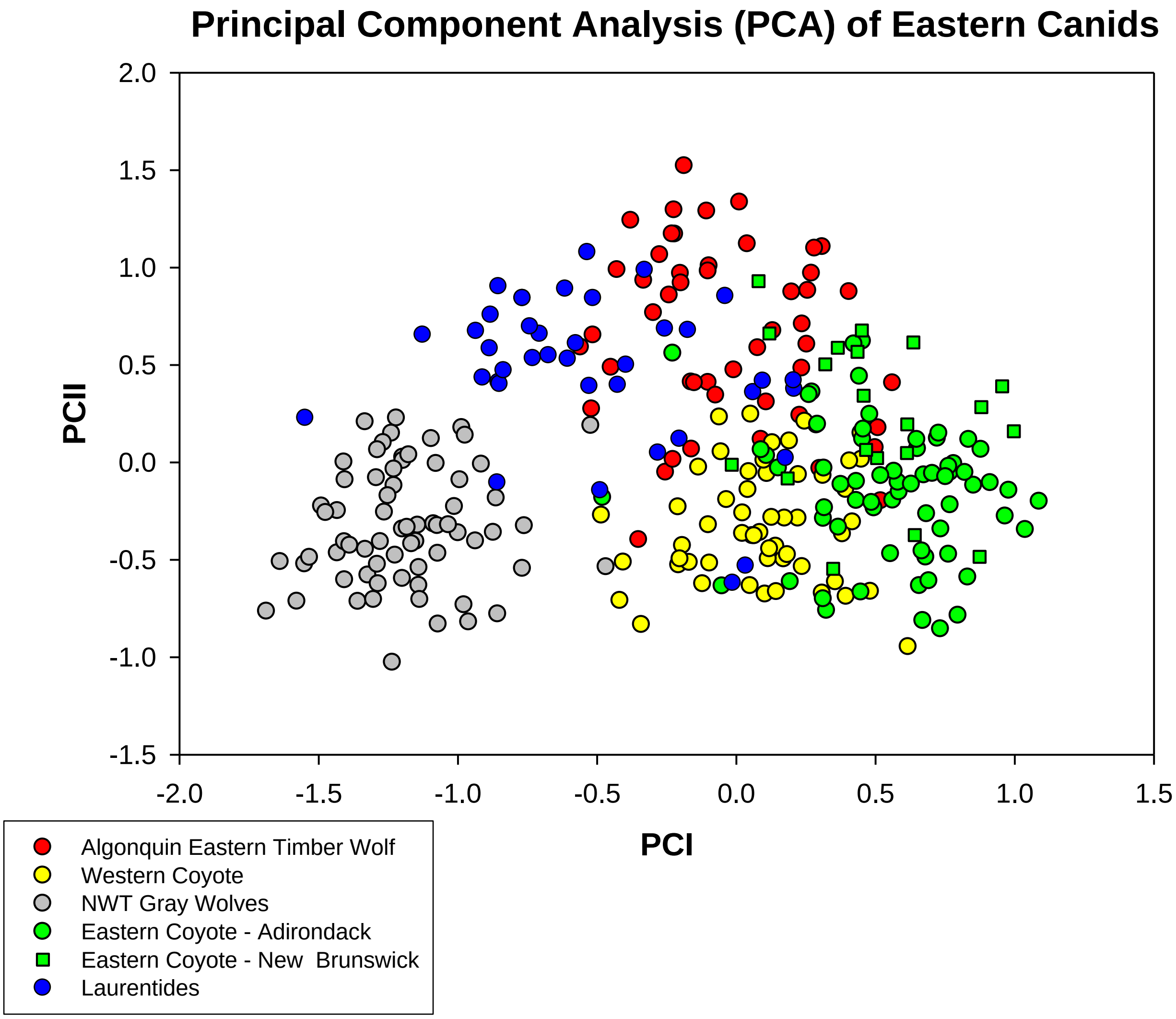
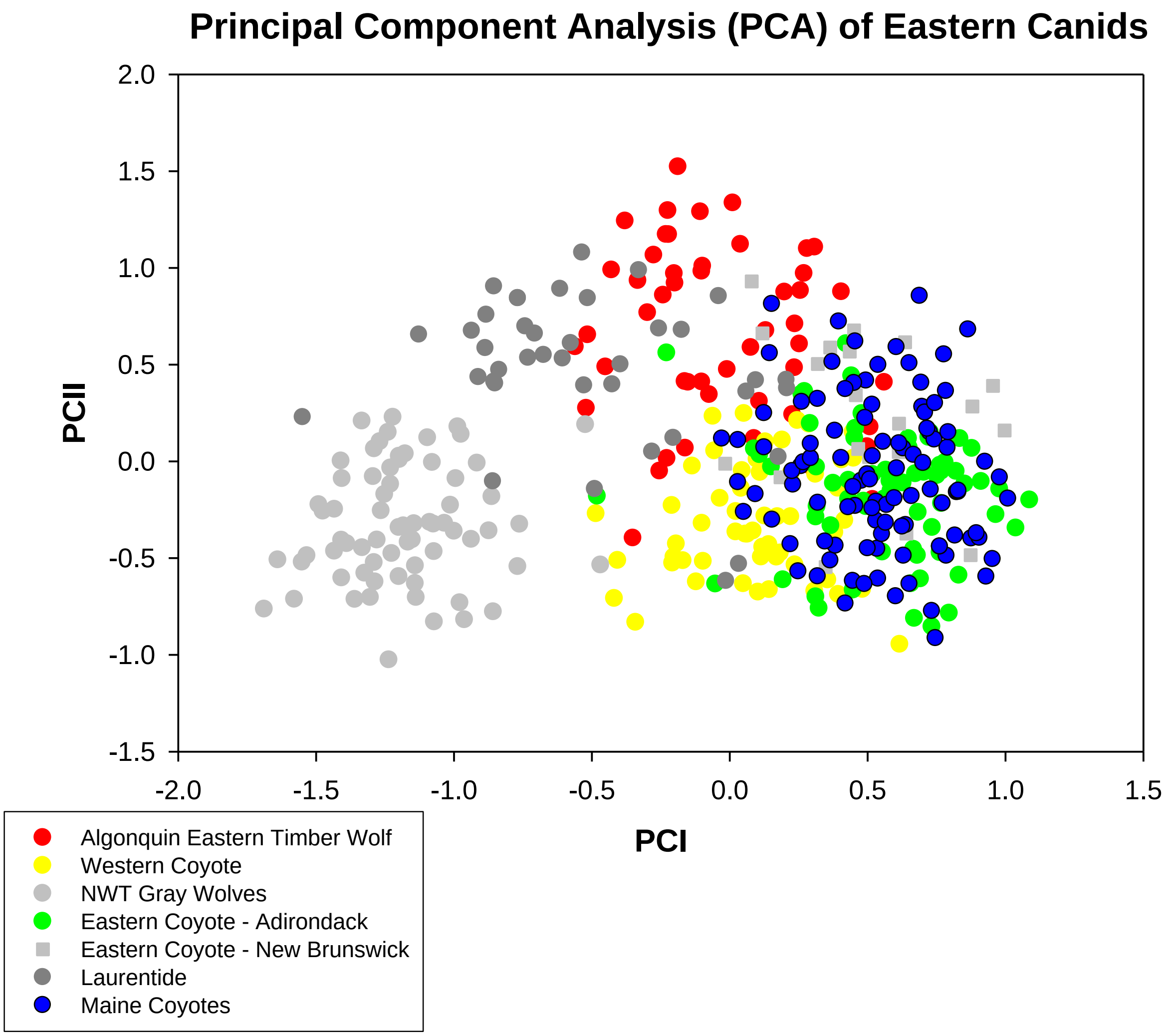


Figure 4b. Principal Component Analysis (PCA) of eastern North American canids including Maine coyotes using 8 microsatellite loci.



Appendix I

Proportion of ancestry of Maine canids assigned to eastern Canadian wolves, western coyotes and eastern coyotes. The program STRUCTURE identifies multi-locus genotypes that are genetically similar without utilizing any known population affiliation, and provides the proportion of ancestry or the ancestry coefficient (q_i) in each cluster. The proportion of ancestry can be thought of as an index for individual animals that describes the average proportion of their genotype that is inferred to come from each cluster (e.g., a cluster may be made up of group of animals with a similar genetic makeup such as one might find in a population).

Maine Sample Number	Eastern Wolf	Western Coyote	Eastern Coyote
1	0.04	0.37	0.59
2	0.02	0.31	0.67
3	0.01	0.01	0.98
4	0.02	0.35	0.63
5	0.03	0.05	0.93
6	0.01	0.01	0.98
7	0.07	0.02	0.91
8	0.01	0.10	0.89
9	0.01	0.01	0.98
10	0.05	0.08	0.87
11	0.23	0.50	0.27
12	0.05	0.03	0.92
13	0.12	0.02	0.86
14	0.06	0.01	0.93
15	0.01	0.02	0.98
16	0.03	0.02	0.95
17	0.32	0.01	0.67
18	0.01	0.06	0.93
19	0.02	0.01	0.96
20	0.02	0.08	0.90
21	0.09	0.35	0.57
22	0.41	0.01	0.58
23	0.01	0.04	0.96
24	0.01	0.01	0.98
25	0.01	0.03	0.96
26	0.01	0.03	0.97
27	0.01	0.08	0.91
28	0.03	0.33	0.65
29	0.10	0.46	0.44

Maine Sample Number	Eastern Wolf	Western Coyote	Eastern Coyote
30	0.06	0.02	0.92
31	0.02	0.51	0.47
32	0.02	0.02	0.96
33b	0.01	0.09	0.90
34(2)	0.01	0.02	0.98
35	0.01	0.06	0.92
36a	0.01	0.02	0.98
36b	0.04	0.02	0.94
37	0.01	0.01	0.99
38	0.04	0.02	0.94
39	0.02	0.01	0.97
40	0.01	0.02	0.98
41	0.01	0.03	0.96
42	0.01	0.02	0.97
43	0.01	0.05	0.94
44	0.04	0.02	0.94
45	0.02	0.03	0.95
46	0.03	0.02	0.94
48	0.02	0.42	0.56
49	0.02	0.08	0.90
50	0.03	0.04	0.93
51	0.01	0.02	0.97
52	0.12	0.14	0.75
53	0.01	0.01	0.98
54	0.01	0.01	0.98
55	0.02	0.02	0.96
56	0.04	0.02	0.94
57	0.01	0.03	0.96
58	0.02	0.02	0.96
59	0.01	0.02	0.97
60	0.04	0.77	0.19
61	0.02	0.01	0.98
62	0.01	0.02	0.98
63	0.01	0.02	0.97
64	0.03	0.01	0.96
65	0.02	0.02	0.97
66	0.01	0.01	0.98
67	0.02	0.09	0.89
68	0.02	0.14	0.84
69	0.09	0.03	0.88

Maine Sample Number	Eastern Wolf	Western Coyote	Eastern Coyote
71	0.02	0.09	0.89
72	0.02	0.02	0.96
73	0.06	0.43	0.51
74	0.01	0.01	0.98
75	0.01	0.02	0.97
76	0.01	0.07	0.92
77	0.01	0.03	0.96
78	0.45	0.01	0.54
79	0.02	0.01	0.96
80	0.02	0.02	0.97
82	0.05	0.04	0.91
83	0.08	0.18	0.74
86	0.02	0.02	0.96
87	0.03	0.06	0.91
88	0.02	0.01	0.97
89	0.01	0.28	0.71
90	0.01	0.02	0.97
91	0.02	0.01	0.97
93	0.89	0.07	0.04
94	0.02	0.58	0.41
96	0.04	0.01	0.95
97	0.54	0.04	0.43
98	0.02	0.02	0.96
99	0.05	0.03	0.92
100	0.03	0.13	0.83
101	0.01	0.03	0.96
102	0.07	0.03	0.90
104	0.02	0.03	0.95
105	0.02	0.01	0.97
106	0.05	0.09	0.86
107	0.01	0.04	0.96

Appendix 2

Morphological measurements of adult canids collected in Maine.

Skull measurements for female coyotes > 1 year in age collected in Maine.

Maine Sample Number	Greatest Length	Zygomatic Width	Alveolar Length maxillary tooth row	Maximum Width Upper Cheek Teeth	Palatal Width	Frontal Shield Width	Height from Toothrow to Orbit	Jugal Depth	Upper Carnassial Crown Length	Upper M2 Crown Width
ME-1	185	101.9	65.9	56.4	21	47.8	28.8	13.2	17.5	10.9
ME-5	183	94.4	52.5	54.5	20.6	48.3	25.8	11	16.6	11.8
ME-9	195	102.9	65.2	59.3	22.3	43.5	28	12.7	17.4	11.7
ME-12	193	94.5	63.8	53.3	21.4	46.9	28.5	12.5	16.9	10.9
ME-13	190	97.2	61.9	53.4	22.4	45.7	27.5	13	15.4	10.6
ME-19	203	100.3	72.4	55.4	21.1	44.1	29.2	14	17.4	11.7
ME-20	189	100.4	67	53.1	19.1	43.4	24.4	12.6	18.1	12.1
ME-21	193	102.6	70.2	57.9	20.7	47.7	25.7	11.5	17.7	12
ME-29	210	109.2	72.2	60.2	20.9	53.5	29.5	13.3	16.3	11.3
ME-42	193	101.9	67	58.6	20.4	46.8	25.9	12.2	18.6	10.7
ME-62	192	96.8	63.6	55	21.5	49.5	27.6	13.3	15.6	11
ME-69	196	96.7	68.5	58.1	21.8	46.7	27.4	12.8	18.2	12.4
ME-70	199	101.3	66.7	58.6	21.8	50.8	27.4	13.5	17.8	11.6
ME-71	188	99	68.8	57.4	18.8	43.8	26.2	12.2	17.4	11.7
ME-73	188	98.2	66.5	56.6	20.1	42.2	23.4	11.4	18.3	11.6
ME-74	198	104.4	68.6	57.8	21	48.9	28.2	13	19.5	12.3
ME-81	197	103.7	70.3	58.1	22.3	46.6	27.3	12.2	17.1	11.4
ME-93	185	102.8	66	58.4	22.3	45.7	27.1	11.7	16.5	11.4
ME-101	185	98.7	66	54.9	21.4	45.1	26	12.8	18.6	10.8
ME-102	190	101.7	66.6	57.8	19.9	49	27	13.2	18.7	11.4
ME-106	196	102.9	67.8	59.1	22.3	53.3	26.4	12.5	17.6	11.2

Skull measurements for male coyotes > 1 year in age collected in Maine.

Maine Sample Number	Greatest Length	Zygomatic Width	Alveolar Length maxillary tooth row	Maximum Width Upper Cheek Teeth	Palatal Width	Frontal Shield Width	Height from Toothrow to Orbit	Jugal Depth	Upper Carnassial Crown Length	Upper M2 Crown Width
ME-6	200	107.9	70	60.8	22.6	47.7	28.4	13.7	18.6	12.7
ME-10	196	105.9	66.7		21.6	46.7	27.5	11.4	17.7	12.7
ME-14	196	105.3	67.9	58.3	20.9	50.1	29.3	14.6	18.8	13
ME-15	197	108.1	68.1	62.3	22.5	48.7	27.3	14.2	20.7	13.5
ME-17		111.6	74.8	61.3	20.5	49.4	29	14.4	19.2	11.7
ME-18	205	105.4	68.8	60	21	43.1	27.1	12.7	19.8	12.2
ME-25	197	107.6	66.3	57.4	20.1	47.2	26.6	13.1	16.8	11.2
ME-27	206	108.7	72.9	61.2	22.3	50.1	28.5	13	18.1	11.3
ME-30	204	107.4	70.5	61.9	22.8	48	30.1	13.3	20.8	13
ME-38	207	106.8	70	58.9	20.6	47.3	28.3	12.3	19.8	11.7
ME-40	189	106.4	68.3	59.8	21.2	55.2	25.8	14.6	20.3	12.5
ME-41	201	109.6	67.1	61.4	24.1	57.1	30.2	15.2	17.7	11
ME-43	217	115.3	76	66.5	25.7	57.8	35.5	18.6	21.8	13.1
ME-45	210	109	69	64.4	24.2	52.8	28.9	15.6	18.2	11.6
ME-49	212	109.9	72.4	62.9	24.2	50.3	31.2	15.1	19.6	13.1
ME-59	190	104.7	63.9	60.5	23.7	52.1	26.8	12.9	18.3	12.1
ME-63	207	103.8	74	61.6	19.7	47.9	27.8	12.9	20.9	12.2
ME-64	206	106.3	70.6	58.1	22.3	50.8	27.8	12.1	18.7	11
ME-65	208	108.9	69.2	59.9	21	54.7	29	14.5	18.4	13.1
ME-78	200	105.3	69.4	59.9	22.1	43.3	27.8	12.7	20.3	11.2
ME-84	210	110.3	71.1		23.4	53.6	32.4	14.4	18.5	11.6
ME-87	210	111.4	74.4	63.4	23	52.9	28.9	14.6	20.7	11.5
ME-96	211	111.7	73.2	62.8	24.7	48.2	28.8	15.1	20.5	12.2
ME-98	211	106	71.6	60.2	22.6	51.5	28.2	14.3	19.5	12.9
ME-99	203	109.7	71.5	61.3	23.4	53.5	29	16.4	20.1	13.1
ME-103	202	106.8	70.9	59.6	21.4	50.6	29.6	13.9	20	12.2
ME-105	188	106.6	66.2	59	20.7	44	28.5	13.7	18.8	11.9
ME-107	197	101.5		58.5		48	28.8	13.2	18.2	11.7

Body measurements of female coyotes > 1 year in age from Maine. All distance measurements are made in millimeters.

Maine Sample Number	Weight kg	Zoological Length	Tail Length	Shoulder Height	Chest Girth	Neck Circum	Head Length	Zygomatic Arch	Ear Length	Nose Pad Width	U. Canine Width	U. Canine Length	Distance Between Tips
ME-1	12.2	1154	315	510	470	290	205	102	101	22.9	9.1	16	33.8
ME-5	12.5	1200	315	520	470	310	197	99	97	23.1	8	17.1	32.7
ME-9	15.2	1246	370	618	476	290	206	125	109	24.6	9.1	20.1	35.7
ME-12	12.5	1175	320	563	460	329	204	98	102	26.2	9.3	15.8	33.6
ME-13	13.3	1157	321	553	476	314	196	91	103	24.3	8.5	17.5	33.9
ME-19	15.4	1227	355	606	497	305	205	99	111	22.3	8.9	14.1	35
ME-20	13.9	1205	340	565	504	279	201	107	108	20.7	9.1	18.7	31.7
ME-21	17.2	1268	365	610	510	315	218	110	107	26.1	9.3	20.8	36.1
ME-29	16.8	1220	350	590	510	325	224	114	115	25.5	8.9	20.6	36.4
ME-42	14.2	1274	345	612	472	300	210	92	101	26.6	9.3	19.5	34.1
ME-62	13.3	1200	312	587	445	290	204	101	103	23.6	7.6	18.4	31.9
ME-69	13.9	1147	310	600	535	350	204	104	108	25.7	9.6	18.9	35.2
ME-70		1222	318	600	515	314	220	105	110	24.5	9.7	17.7	35.3
ME-71	14.1	1163	321	605	490	300	187	93	105	26	8.8	18.5	33.6
ME-73		1119	300	575	462	282	198	95	93	19.7	8.8	18.3	32.7
ME-74	15.5	1199	317	610	550	282	203	108	100	27.9	9.6	20.1	35.8
ME-81	13.9	1262	372	620	515	290	208	103	107	25.3	9.4	20.1	35.9
ME-93	12.6	1158	321	595	480	310	201	106	107	23.4	8.6	18.8	33.5
ME-101	12.8	1150	310	560	550	310	193	94	109	23.3	9.1	19.7	33.2
ME-102	15.2	1217	345	585	470	300	184	101	110	23.7	10.3	20.5	35.4
ME-106	16.0	1220	345	605	520	310	216	106	103	22.5	8.7	14.3	33.8

Continuation of body measurements of female coyotes > 1 year in age from Maine. All distance measurements are made in millimeters.

Maine Sample Number	L. Canine Width	L. Canine Length	Distance Between Tips	Front R. Heel-pad Width	Front R. Heel-pad Length	Total Front R. Foot Length	Total Hind Foot Length	2nd Thoracic Nipple Width	2nd Thoracic Nipple Height	Vulva Width	Vulva Length
ME-1	8.6	16.3	31.2	27.6	25.8	70	205	2.67	4.56	4.2	12.5
ME-5	8.3	16.2	29.5	25.9	20.6	65	168	3.7	3.9	6.3	11.9
ME-9	7.9	17.8	32.1	30.1	29.1	80	203			4.4	7.7
ME-12	7.2	15.6	28.9	27.6	23.1	79	193	3.7	6	4.2	12.7
ME-13	7.7	16.3	30.6	28.5	24	60	177			3.5	9.7
ME-19	8.3	16.9	31.1	30.1	31.4	84	210			1.7	14.2
ME-20	8.6	13.6	27.8	27.9	26.3	80	200	2.9	6.2	2.3	8.7
ME-21	9.5	18.2	33.8	28.9	30.4	81	210			1.8	10.3
ME-29	7.5	17.3	32.3	31.6	32.5	86	203	3.3	5.9	4.3	13.8
ME-42	9.3	18.1	29.9	27	27.8	79	210	2.6	5.3	2.4	14.2
ME-62	8.3	16.5	28.9	29.9	24.5	81	192	6.3	5.8	3.7	18.9
ME-69	8.6	18.3	31.6	28.7	25.8	75	212	3.6	5.2	3.5	8.6
ME-70	9.2	17.3	31.8	31.7	30.9	83	200	3.7	6.4	2.7	6.9
ME-71	8.9	16.3	27.9	28.8	26.4	80	190	2.1	3.2	2.3	13.3
ME-73	8.3	16	28.3	23.3	23.8	70	182			3.5	9.4
ME-74	9.4	18.1	31.4	32.5	28.6	79	203			2.8	9.6
ME-81	10.4	16.5	31.6	31.7	29.1	80	202			4.7	10.8
ME-93	8.5	17.2	28.9	29.5	25	73	200	2.5	3.8	4.1	13.4
ME-101	8.5	17.8	29.8	29.4	25.7	80	202			2.8	9.9
ME-102	9.6	18	32	29.6	22.7	77	205			3.8	10.7
ME-106	10.3	14.5	28.1	27.2	25.8	79		3.6	3.5	4.6	11

Body measurements of male coyotes > 1 year in age from Maine. All distance measurements are made in millimeters.

Maine Sample Number	Weight kg	Zoological Length	Tail Length	Shoulder Height	Chest Girth	Neck Circum	Head Length	Zygomatic Arch	Ear Length	Nose Pad Width	U. Canine Width	U. Canine Length	Distance Between Tips
ME-6	17.5	1260	285	570	560	360	214	111	109	26.3	10.6	22.2	36.6
ME-10	11.2	1215	355	600	454	290	209	97	100	25.1	9.2	20.6	34.9
ME-14	18.5	1288	395	630	590	350	209	129	109	22.4	8.3	19.2	34.3
ME-15	16.2	1215	325	565	530	370	202	103	100	27.7	9.7	22.1	36.5
ME-17	20.2	1284	335	625	634	350	217	113	110	25.37	10.4	22.8	36.6
ME-18	17.1	1234	360	600	526	309	217	108	110	26.1	9.7	21	36.7
ME-25	16.3	1224	350	605	490	305	203	93	105	26.4	8.7	19.6	33.2
ME-27	16.3	1262	320	595	495	320	223	108	111	29	8.1	19.6	36.2
ME-30	17.7	1227	340	600	512	340	214	103	110	23.9	10.6	20.7	37.4
ME-38	13.7	1265	343	640	485	325	215	113	104	24.9	9.5	20.7	33.9
ME-40	11.5	1168	340	605	487	290	201	108	94	23.8	10	19.3	34
ME-41	17.1	1250	345	620	570	330	204	114	104	26.3	8.6	20.9	37.9
ME-43	21.4	1279	320	640	630	370	228	120	110	25.5	10.5	20.9	42.4
ME-45	18.9	1309	342	655	532	350	226	115	115	25	10.5	22.1	40.8
ME-49	18.3	1289	380	675	570	340	224	116	104	26.4	10	22.2	41.1
ME-59	17.2	1147	297	610	580	365	200	109	99	26.9	9.8	18.6	26.2
ME-63	15.4	1165	330	625	530	320	222	104	102	25	10	21.5	34.8
ME-64	15.8	1307	350	627	483	362	215	99	99	26.6	10	19.4	
ME-65	16.7	1204	321	609	580	400	224	109	103	27.4	9.9	19.9	37
ME-78	14.5	1208	333	610	500	310	213	96	106	22.8	9.7	21.5	35.4
ME-84	15.9	1275	365	640	558	340	216	116	105	25.9	9.7	20.4	38.3
ME-87	16.8	1265	355	623	550	340	219	110	105	29.7	9.4	19.4	40.9
ME-96	20.6	1328	395	655	610	340	225	116	107	25.1	10.8	24.1	37
ME-98	17.0	1270	357	637	540	330	220	111	115	24.6	9.2	21.3	36.3
ME-99	17.5	1302	375	650	540	320	207	117	110	27.8	9.6	23.5	36.5
ME-103	16.6	1275	360	615	520	310	218	101	110	26	9.1	24.4	35.3
ME-105	15.3	1195	360	565	576	330	201	107	105	26	9.1	22.1	35.7
ME-107	14.5	1235	360	635	540	320	201	102	104	22.7	10	22	25.1

Continuation of body measurements of male coyotes > 1 year in age from Maine. All distance measurements are made in millimeters.

Maine Sample Number	**L. Canine Width**	**L. Canine Length**	**Distance Between Tips**	**Front R. Heel-pad Width**	**Front R. Heel-pad Length**	**Total Front R. Foot Length**	**Total Hind Ft Length**
ME-6	9.5	19.1	32.8	30.2	26.3	80	200
ME-10	9.2	18.4	30.9	29.6	23.2	75	190
ME-14	8.9	17.4	34.4	29.8	26.9	80	210
ME-15	7.9	17.7	31.4	30.6	27.1	80	190
ME-17	10.7	19.6	33.1	31.1	28.6	91	205
ME-18	9.1	17.6	32.1	29.8	31.3	82	205
ME-25	8.1	15.5	28.9	30.2	27.7	77	200
ME-27	8.2	16.9	31.7	36	34	82	207
ME-30	9.8	18.2	33.4	29.6	31.2	85	207
ME-38	9	17.9	30.8	31.6	32.8	80	212
ME-40	9.5	17.6	30.1	28.3	26.1	71	194
ME-41	9.4	17.1	31.7	26.5	31.9	82	220
ME-43	10.9	17.1	35.3	34.9	33.3	84	212
ME-45	9.7	20.1	35	31.4	35.5	80	212
ME-49				31	32.8	83	220
ME-59	9.5	17.8	32.1	30.8	27.7	81	190
ME-63	9.7	20	31.3	28.1	28.2	79	191
ME-64	9.9	18.9	33.3	30.7	32.6	75	203
ME-65	10.4	18.8	34.3	32.1	30.1	82	195
ME-78	10.3	19.5	31.4	26.2	26.1	77	192
ME-84	10	16.4	33	31.9	29.7	80	220
ME-87	9.3	20.4	34.1	30.8	32.1	77	208
ME-96	9.5	18.9	34.1	33.4	33.1	85	228
ME-98	9.3	18.3	30.6	32.2	30.9	82	217
ME-99	9.2	18.2	31.8	31	29.3	81	222
ME-103	8.2	19.1	31.3	31.2	28.3	75	192
ME-105	9.5	20.9	32.3	29.5	27.7	79	195
ME-107	10	18	30.8	30.5	27.5	79	225

Appendix 3

Museum Search for Historical Wolf Specimens from New England

Museums Contacted in Search of Historical New England Wolf Specimens
by the Maine Department of Inland Fisheries and Wildlife

MUSEUM	TOWN	STATE	PHONE #	E-MAIL
Maine State Museum	Augusta	ME	(207) 287-2301	
The Nylander Museum	Caribou	ME	(207) 493-4474	
Natural History Museum	Bar Harbor	ME		
University of Maine, Farmington	Farmington	ME	(207) 778- 7361	barker@maine.edu
Little Nature Museum	Weare	NH	(603) 529-7180	
University of New Hampshire	Durham	NH	(603) 862- 4749	mps@christa.unh.edu
New England Museum Association	Boston	MA	(617) 242-2283	www.nemanet.org
Museum of Science	Boston	MA	(617) 723-2500	information@mos.org
Discovery Museums	Action	MA	(508) 364-4201	
Robert Cole Museum of Natural History	Holyoke	MA	(413) 527-4805	
Springfield Science Museum	Springfield	MA	(413) 263-6800	
Westfield State College,	Westfield	MA		dlovejoy@wisdom.wsc.ma.edu
Boston University Museum and Herbarium	Boston	MA	(508) 289- 7499	mmccaff@bu.edu
Montshire Museum of Science	Norwich	VT	(802) 649-2200	montshire@montshire.org
Fairbanks Museum & Planetarium Collection	St.Jonesbury	VT		peggy@connriver.net
Carnegie Museum of Natural History	Pittsburgh	PA	(412) 665-2615	lagiovaned@carnegiemuseums.org
The Academy of Natural Sciences	Philadelphia	PA	(215) 299- 1078	webmaster@acnatsci.org egilmore@acnatsci.org
American Museum of Natural History	New York	NY	(212) 769- 5100	
Cornell University, Vertebrate Collections	Ithaca	NY		kjm2@cornell.edu
Buffalo Museum of Science	Buffalo	NY		rbrew@sciencebuff.org
New York State Museum	Albany	NY		nysmpress@mail.nysed.gov
Roosevelt Wildlife Collection	Syracuse	NY		wfporter@eesf.edu

The NJ State Museum		NJ		feedback@sos.state.nj.us
The Newark Museum		NJ		publicrelations@newarkmuseum.org
Mashantucket Pequot Museum	Mashantucket	CT	1-800-411-9671	
Stamford Museum and Nature Center	Stamford	CT	(203) 322-1646	
Peabody Museum of Natural History	New Haven	CT	(203) 432-5050	
Bruce Museum	Greenwich	CT	(203) 869- 0376	
University of Montana, Zoological Museum	Missoula	MT		dchristi@slway.umt.du
California Academy of Sciences	San Francisco	CA	(415) 750- 7346	kcebra@calacedmy.org
Natural History Museum of LA County	Los Angeles	CA	(213) 763- 3404	info@nhm.org
Pacific Grove Museum of Natural History	Pacific Grove	CA	(831) 648- 5716	pgmuseum@mbay.net
University of Connecticutt	Storrs	CT	(203) 486- 4457	dubos@uconnvm.uconn.edu
University of Delaware	Newark	DE		jbowman@udel.edu
US National Museum of Natural History	Washington	DC	(202) 357- 2150	mnhvz049@sivm.si.edu
The Field Museum	Chicago	IL		fooden@fieldmuseum.org
Kansas State University	Manhatten	KS	(913) 532- 6622	dwkaufma@lter-konza.konza.ksu.edu
Zazock Thompson Natural History	Burlington	VT		wkilpatr@moose.uvm.edu
University of Georgia, Museum of Natural History	Athens	GA		kcarlin@arches.uga.edu
European Specimens- Paula Jenkins				pdj@nhm.ac.uk

Listing of Museum Collections Compiled by Ron Nowak, Smithsonian Institution (personal communication)

Paleontological and Archeological Specimens Examined

This list provides details for series from the region of interest, thought to date prior to AD 1800. Specific identifications are as determined in this study. Information on a few additional individuals is given in the text. Abbreviations used are: ANSP, Academy of Natural Sciences, Philadelphia; AMNH, American Museum of Natural History; CM, Carnegie Museum; CNM, National Museum of Canada; FGS, Florida Geological Survey; ILSM, Illinois State Museum; INSM, Indiana State Museum; MCZ, Harvard University Museum of Comparative Zoology; MSU, Michigan State University Museum; MVZ, University of California Museum of Vertebrate Zoology; NCSU, North Carolina State University Department of Zoology; PU, Purdue University Department of Forestry and Conservation; ROM, Royal Ontario Museum; UAR, University of Arkansas Department of Zoology; UF, Florida Museum of Natural History; UMI, University of Michigan Museum of Zoology; UMN, University of Minnesota Museum of Natural History; USNM, United States National Museum.

CANIS LEPOPHAGUS.-- FLORIDA: Santa Fe River 1B, Gilchrist County, late Blancan, five mandibular fragments—UF 10423, 10424, 10836, 10837, 10858, mandible—collection of D. Damrow, Mosinee, Wisconsin; Haile 12B, Alachua County, mandibular fragment—UF.

CANIS LATRANS.-- FLORIDA: Devil's Den, Levy County, late Rancholabrean (c. 10,000 ybp), three mandibular fragments—UF 11514, 11515, 11517; Melbourne, Brevard County, late Rancholabrean (c. 10,000 ybp), rostral fragment—MCZ 5909, mandibular fragment—USNM 12947; Vero (stratum 3), Indian River County, late Rancholabrean, maxilla—FGS 7036; Cutler site, near Perrine, Dade County, maxillary fragment—UF 143286, mandibular fragment—UF 143279. INDIANA: Megenity Peccary Cave, Crawford County, late Rancholabrean (c. 33,500 ybp), cranium and maxillae—INSM 71-3-62-5-1, two maxillary fragments—INSM 71-3-60-5-6, 71-3-63-5-24, mandible—INSM 71-3-3-60-5-1, four mandibular fragments—INSM 71-3-62-5-59, 71-3-62-5-84, 71-3-62-5-85, 71-3-62-5-96, four P4—INSM 71-3-62-5-44, 71-3-62-5-66, 71-3-63-5-22, 71-3-63-5-43, three M1—INSM 71-3-62-5-129, 71-3-62-5-142, 71-3-62-5-129, three m1—INSM 71-3-62-5-25, 71-3-62-5-117, 71-3-62-5-140. MARYLAND: Cumberland Cave, Allegany County, early Rancholabrean, mandibular fragment—USNM. PENNSYLVANIA: Frankstown Cave, Blair County, late Rancholabrean (c. 14,000 ybp), two mandibular fragments from same individual—CM 11027. VIRGINIA: stone quarry 1 km NW Edinburgh, Shenandoah County, early Rancholabrean, maxillary fragment—USNM. WEST VIRGINIA: New Trout Cave, Pendleton County, late Rancholabrean (10,000--40,000 ybp), p4—USNM.

CANIS PRISCOLATRANS _(= *CANIS EDWARDII*).-- FLORIDA: Haile 12A, Alachua County, late Blancan, cranial fragment—UF 11516; Haile 21A, Alachua County, early Irvingtonian, rostral fragment (cast)—UF 62561, mandible—UF 63175, three mandibles (casts)—UF 62562, 62563, 62564, four mandibular fragments—UF

62568, 63174, 63527, 62565, two P4—UF 18049, 124537, M1—UF 63623, two p4—UF 63311, 124539, m1—UF 62567; Inglis 1A, Citrus County, early Irvingtonian, three maxillary fragments—UF 18046, 18047, 67846, two mandibular fragments—UF 19323, 19324, P2—UF 18050, P4—UF 18049, two M1—UF 19405, 19406, two M2—UF 18048, m1—UF 19404; Crystal River Power Plant, Citrus County, early Irvingtonian, maxillary fragment—UF17074; Phosphoria Mine, Polk County, early Irvingtonian, maxillary fragment (cast)—UF 58332; Leisey Shell Pits, Hillsborough County, early Irvingtonian, cranial fragment—UF 67092, seven maxillary fragments—UF 81654, 81655, 81663, 81664, 81665, 81666, 124531, mandible—UF 63667, three mandibular fragments—UF 64399, 87283, 95647, four P4—UF 80662, 81656, 81661, 81668, two M1—UF 81657, 81669, p2—UF 81675, p3—UF 81674, two p4—UF 81658, 81659, six m1—UF 81660, 81662, 81672, 81673, 84752, 87285, two m2—UF 81689, 87297; Rigby Shell Pit, Sarasota County, maxillary fragment—UF 40090, mandibular fragment—UF 40091; Punta Gorda, Charlotte County, early Irvingtonian, mandibular fragment—UF 36429. PENNSYLVANIA: Port Kennedy deposit, Montgomery County, early Irvingtonian, P4, M1, M2, and p4—ANSP 57-58.

CANIS ARMBRUSTERI.-- ARKANSAS: Conrad Fissure, Newton County, early Rancholabrean, cranial fragment, isolated teeth—AMNH 11761, mandibular fragment—AMNH 11762. FLORIDA: Haile 7A, Alachua County, early Rancholabrean, maxillary fragment and pair of mandibles—UF 11845; McLeod lime rock mine, Levy County, middle Irvingtonian, cranial fragment—AMNH 67286, two maxillary fragments (probably from same individual)—AMNH 67287-67288, two mandibular fragments (probably from same individual)—AMNH 67289-67290, mandibular fragment—AMNH 67291; Coleman 2A Local Fauna, Sumter County, late Irvingtonian, near-complete skull without mandibles—UF 11519, cranial fragment, maxillary fragment, and three mandibular fragments—UF 11520, mandibular fragment and two m1—UF 12121, mandibular fragment—UF 11518, two P4—UF 12114. MARYLAND: Cumberland Cave, Allegany County, early Rancholabrean, two skulls with mandibular fragments—USNM 8144, 11881, six skulls—USNM 7994,11883, 11885, 11886, 11887, 12288, 13 mandibular fragments—USNM 7482, 7661, 8144, 8168, 8169, 8172, 11881, 11882, 11888, 12290, 12291, 12293, 12295.

CANIS DIRUS (all late Rancholabrean).-- FLORIDA: Ichetucknee River, Columbia County, maxillary fragment—UF 8006, three mandibular fragments—UF 8005, 12899, 17717; Hornsby Springs, Alachua County, maxillary fragment—UF 3988, mandibular fragment—UF 3987; Devil's Den, Levy County, incomplete skull—UF 7996; Wekiva River, Levy County, mandibular fragment—UF 14204; Reddick 1A, Marion County, crushed skull with mandibles—UF 2923, two crushed skulls without mandibles—UF 3081 and unnumbered, mandibular fragment—UF, isolated P4, two M1, M2, and m1—UF; Eichelberger Cave, Marion County, two mandibular fragments (probably from same individual)—UF 1622, 1623; Melbourne, Brevard County, mandible—USNM 12946, two isolated P4, two M1, and four m1—USNM; Seminole Field, Pinellas County, mandibular fragment, AMNH 23568, M1—AMNH 23582, M2—AMNH 23569, two m1—AMNH 23565, 23567; Bradenton, Manatee County, maxillary fragment—UF 3276, mandibular fragment—UF 2259; Vero (stratum 2), Indian River

County, skull without mandibles—FGS 7166; Cutler site, near Perrine, Dade County, mandibular fragment—UF 156956, p4 and m1—UF 135887 . GEORGIA: Ladds, Bartow County, M1—USNM 23698. INDIANA: Ohio River, Vanderburgh County, maxillary fragment—ANSP 11614. KENTUCKY: Welsh Cave, Woodford County, cast of skull without mandibles—CM 12625, cast of mandible from different individual—CM 12625a. PENNSYLVANIA: Frankstown Cave, Blair County, maxillary fragment—CM 11023, three mandibular fragments—CM 11022, 11024, 11026. West Virginia: Rennick, Greenbrier County, mandible—CM 24327.

CANIS RUFUS.-- ALABAMA: Crow Island Indian midden, Jackson County, c. 1,000 ybp, mandible—UMI 91100. ARKANSAS: Eddy Bluff shelter, Washington County, early Recent, maxillary fragment—UAR; Banks Site, Crittenden County, c. 425 ybp, mandible—ILSM. FLORIDA: Devils's Den, Levy County, late Rancholabrean (c. 10,000 ybp), cranial fragment—UF 16397, mandible—UF 11513; Withlacoochee River, Citrus County, late Rancholabrean (c. 10,000 ybp), m1—collection of D. Wells, Arlington, Virginia; Jungerman Site, Indian River County, c. 250 ybp, m1, m2—UF; Melbourne, Brevard County, late Rancholabrean (c. 10,000 ybp), mandibular fragment—MCZ 17789; Nichol's Hammock, Dade County, c. 200 ybp, mandible—UF 16711. ILLINOIS: Litchfield, Montgomery County, late Rancholabrean, collection of D. Damrow, Mosinee, Wisconsin; Palestine site, Crawford County, c. 2,000 ybp, maxillary fragment—ILSM CW4F67, mandibular fragment—ILSM CW4F66. MARYLAND: Doepkin's Farm site, c. 300 ybp, Anne Arundel County, maxillary fragment—seen at site, current location unknown. NEW YORK: Garoga site, Fulton County, c. 400 ybp, maxillary fragment and two mandibular fragments—CM G-837. NORTH CAROLINA: Franklin site, Macon County, c. 300 ybp, maxillary fragment—NCSU. OHIO: Blain site, Ross County, maxillary fragment—ILSM. PENNSYLVANIA: Johnston site, Indiana County, c. 350 ybp, maxillary fragment—CM 802; Hartley site, Greene County, c. 500 ybp, mandibular fragment—CM 4531; New Paris Sinkhole No. 2, Bedford County, c. 1,900 ybp, incomplete skull and pair of mandibles—CM 6548a, 6548b; Eschelman site, Lancaster County, c. 350 ybp, cranial fragment, pair of mandibles, and three mandibular fragments—CM 36 La 12. TENNESSEE: Citico Mound, Hamilton County, c. 1,000 ybp, mandible—USNM 200145. WEST VIRGINIA: Buffalo Village site, Putnam County, c. 300 ybp, three mandibular fragments and isolated m1—CM 46 Pu 31; Mount Carbon site, Fayette County, c. 500 ybp, m1—CM 46 Fa 7; Piercy's Cave, Greenbrier County, late Rancholabrean, m1—USNM.

Specimens Used in Multivariate Analyses

This list provides information on those series subjected to canonical discriminant analysis. Greater detail is given for specimens from the region of interest. Specific and subspecific identifications are as determined in this study. Details on a few additional individuals, also used in multivariate analysis, are provided in the text.

CANIS LATRANS, western.-- All from Colorado and Idaho, all in USNM.

CANIS LUPUS, western.-- The six series correspond to six originally designated subspecies (Goldman 1944; Hall 1981): *irremotus* (14 specimens, all in USNM), Idaho, Montana, Wyoming; *youngi* (28, all in USNM), Colorado, New Mexico, Utah, Wyoming; *mogollonensis* (17, all in USNM), Arizona, New Mexico; *monstrabilis* (7, all in USNM), central and western Texas; *nubilus* (7, USNM; 1, AMNH), Nebraska, Kansas, Oklahoma; *lycaon* (15, USNM; 8, UMN; 5, PU), Minnesota, Michigan (Isle Royale only). The Minnesota and Isle Royale specimens, as well as all the others, are now thought to represent the single subspecies *C. lupus nubilus* (Nowak 1995).