Genetic Characterization of Eastern "Coyotes" in Eastern Massachusetts

Jonathan G. Way[1], Linda Rutledge[2], Tyler Wheeldon[2],
and Bradley N. White[2]

Abstract - This study examined the genetic nature and relatedness of *Canis latrans* (Coyotes) in eastern Massachusetts (i.e., eastern Coyotes). We characterized 67 animals at the mitochondrial DNA control region, and 55 of those at 8 microsatellite loci. Structure analysis and factorial correspondence analysis of the microsatellite genotypes indicated that the eastern Coyotes in Massachusetts clustered with other northeastern *Canis* populations and away from western Coyotes, *C. lycaon* (Eastern Wolves), and *C. lupus* (Gray Wolves). They contained mitochondrial haplotypes from both western Coyotes and Eastern Wolves, consistent with their hybrid origin from these two species. There was no evidence of either *C. lupus familiaris* (Domestic Dog) or Gray Wolf mitochondrial DNA in the animals. These results indicate that the eastern Coyote should more appropriately be termed "Coywolf" to reflect their hybrid (*C. latrans* x *lycaon*) origin. Genetic data were also used to assess parental and kinship relationships, and confirmed that family units typically contain an unrelated breeding pair and their offspring. Lastly, a synthesis of knowledge of the eastern Coyote as well as implications for Wolf recovery in the northeast US is provided.

Introduction

Canis latrans Say (Coyotes) living in northeastern North America (i.e., eastern Coyotes) have been an enigma to both scientists and laypeople for many years (Parker 1995). This wild canid started to appear in northern New England and New York in the 1930s and 1940s and currently inhabits all of the northeastern United States and southeastern Canada, ranging from wilderness to urban areas (Fener et al. 2005, Parker 1995). The animals are often described as a big version of the western Coyote or a small Wolf, and many northern New Englanders still call them "coy-dogs" (Way 2007), yet there remains speculation regarding its origins (Wilson et al. 2009). While the eastern Coyote has been confirmed as the largest version of the species (Gompper 2002, Lawrence and Bossert 1969, Silver and Silver 1969, Way 2007, Way and Proietto 2005), the animal's large body size has confused its taxonomy (i.e., the var. indicates a variation of Coyote) since it was first described by Lawrence and Bossert (1969) and Silver and Silver (1969).

Hypotheses as to why eastern Coyotes are bigger include response to enhanced food supply or larger prey (Thurber and Peterson 1991), genetic adaptation to prey, mainly *Odocoileus virginianus* Boddaert (White-tailed Deer) (Larivière and Crête 1993), or their being Coyote-dog hybrids (Mengel 1971).

[1]Eastern Coyote Research, 89 Ebenezer Road, Osterville, MA 02655. [2]Natural Resources DNA Profiling and Forensic Centre, Environmental and Life Sciences Graduate Program, Trent University, Peterborough, ON L8S 4K1, Canada. [*]Corresponding author - jw9802@yahoo.com.

Most of the data reject these hypotheses since medium-sized food (i.e., mice and rabbits) and deer are abundant throughout the United States (US) (discussed in Way 2007), and coy-dogs reproduce in fall and give birth in winter instead of mating in winter and giving birth in early spring as wild canids do (Mengel 1971, Way et al. 2001). The asymmetry of coy-dog versus wild canid (i.e., eastern Coyote) reproduction cycles appears to be an effective barrier preventing introgression of dog genes into wild canid populations in northeastern North America despite it occurring historically in the southeast US (e.g., Adams et al. 2003a)—this difference is likely due to harsh winters in the north, which prevent coy-dogs from surviving when born in mid-winter.

Canis lycaon Schreber (Eastern Wolves) in central Ontario, Canada, are genetically similar to and probably the same species as *C. rufus* Audubon and Bachman (Red Wolf) (Kyle et al. 2006, Wilson et al. 2000). The conspecific nature of Eastern and Red Wolves is supported by an accumulation of genetic evidence (e.g., Kyle et al. 2006, 2008; Wilson et al. 2000, 2003, 2009). Therefore, to simplify, we hereafter use Eastern Wolves (*C. lycaon*) as an umbrella termninology that includes Red Wolves (*C. rufus*), although we note that Red Wolf samples from the southeastern US were not analyzed in this study. Evolutionarily, this small deer-eating wolf (Theberge and Theberge 2004) is more closely related to Coyotes than to *C. lupus* L. (Gray Wolf) (Hedrick et al. 2002, Wilson et al. 2000). The Eastern Wolf (not the Gray Wolf) is believed to be the original *Canis* species historically present in northeastern North America (Kyle et al. 2006, 2008; Wilson et al. 2000, 2003, 2009; although see Nowak 2002) before being extirpated by humans, and is likely the wolf (at a very small population size) that would have hybridized with western Coyotes during their eastward migration in the early 1900s (Parker 1995). The close evolutionary relationship of *C. latrans* and *C. lycaon* probably facilitated hybridization following landscape change, especially when wolf numbers were low (Grant and Grant 1997) in areas such as southern Ontario. In fact, the biggest perceived threat currently facing Eastern Wolves in the southeast US is hybridization with Coyotes colonizing the periphery of the North Carolina recovery area (Adams et al. 2003b). However, even small (i.e., re-colonizing) populations of Gray Wolves in the western US show no evidence of hybridization with western Coyotes (e.g., Pilgrim et al. 1998).

The objectives of this study were to: (1) characterize the genetic composition of Massachusetts eastern Coyotes in relation to other groups of Coyotes and wolves from the US and Canada, and (2) determine parentage and kinship within putative family units. We tested the hypotheses that: (1) eastern Coyotes in Massachusetts were hybrids between Eastern Wolves and western Coyotes, and (2) these animals formed social groups (packs) consisting of unrelated breeding pairs and their offspring.

Methods

Samples

Eastern Coyotes were sampled from Cape Cod (specifically, in and around the town of Barnstable) and within 20 km of Boston, MA (*n* = 67). Whole blood

was obtained by venipuncture of live-trapped individuals that were subsequently released (e.g., Way 2007). Tissue (ear) or organ samples (liver, muscle) were taken opportunistically from dead animals. Previously analyzed samples representative of western Coyotes (Texas), Eastern Wolves (Algonquin Provincial Park), Gray-Eastern Wolf hybrids (northeastern Ontario and Quebec), and Gray Wolves (Northwest Territories) were included for the genetic analyses. These sample groups were assigned a species or hybrid designation based on a combination of mitochondrial DNA (mtDNA) and microsatellite data (and some Y-chromosome data) from previous studies (Grewal et al. 2004; Wheeldon and White 2009; Wilson et al. 2000, 2003, 2009).

To be consistent with Way (2007), we classified eastern Coyote range as living in established populations in northeastern North America east of longitude 80° (recent range expansion described by Parker [1995] as New England, New York, New Jersey, Pennsylvania, Ontario, and Quebec). Although seemingly arbitrary, this line is useful because it delineates where larger "Coyotes" occur (Way 2007, Way and Proietto 2005) and where they have been recently documented (Fener et al. 2005, Parker 1995).

DNA extraction, amplification, and genotyping

All samples were extracted with a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Mississauga) using the manufacturer's protocol. A 343–347 base pair (bp) fragment of the mtDNA control region was amplified using primers AB13279 (5'-GAA GCT CTT GCT CCA CCA TC-3'; Pilgrim et al. 1998) and AB13280 (5'-GGG CCC GGA GCG AGA AGA GGG AC-3'; Wilson et al. 2000). This region allows differentiation between Old World sequences (i.e., Gray Wolves [*C. lupus*] or Dogs [*C. lupus familiaris* L.]) and New World sequences (i.e., Eastern Wolves [*C. lycaon*] or Coyotes [*C. latrans*]), and also differentiates between haplotypes commonly found in present day Coyotes and those found in Eastern Wolves (Wilson et al. 2000, 2003). PCR products were cleaned with ExoSap-IT (USB Corporation, Cleveland, OH) prior to sequencing on a MegaBACE 1000 (GE Healthcare, Quebec, QC, Canada). We edited, aligned, and compared sequences to known haplotypes in Bioedit (Hall 1999), and haplotypes were assigned based on a 230-bp region (Wilson et al. 2000). Gender was confirmed by amplification of the zinc finger intron (Shaw et al. 2003). We attempted amplification of 8 nuclear microsatellite loci for each sample (cxx225, cxx200, cxx123, cxx377, cxx250, cxx204, cxx172, cxx109; Ostrander et al. 1993, 1995). Amplified products were analyzed on a MegaBACE 1000, and alleles were scored in GENEMARKER v1.7 (SoftGenetics LLC).

Data analysis

Genetic analysis. We analyzed microsatellite genotype data using STRUCTURE v2.2 (Falush et al. 2003, 2007; Pritchard et al. 2000), including genotypes of samples from this study (Massachusetts: *n* = 55) and others based on the same 8 loci (Grewal 2001, Wilson et al. 2009), as well as some previously unpublished data generated by the Natural Resources

DNA Profiling and Forensic Centre (NRDPFC) at Trent University: Northwest Territories ($n = 65$); Northeastern Ontario ($n = 33$); Quebec ($n = 37$); Algonquin Provincial Park ($n = 49$); Frontenac Axis ($n = 74$, located in southeastern Ontario between Algonquin Park and the Adirondacks); Adirondack State Park ($n = 66$); Cortlandville, NY ($n = 24$); Maine ($n = 101$); New Brunswick ($n = 20$); Ohio ($n = 15$); North Carolina ($n = 22$); and Texas ($n = 22$) (P. Wilson, Trent University, Peterborough, ON, Canada, W.J. Jakubas, Maine Department of Inland Fisheries and Wildlife, Bangor, ME, and S. Mullen, University of Maine, Orono, ME, 2004 unpubl. data; a copy of the unpublished report is available from W.J. Jakubas). The admixture model of STRUCTURE was run for K = 1 to K = 10 with five repetitions of 10^6 iterations following a burn-in period of 250,000 iterations for each K. The F-model (i.e., correlated allele frequencies) and I-model (i.e., independent allele frequencies) of STRUCTURE were both implemented to compare results, and a separate alpha was inferred for each population to account for asymmetric admixture. We computed the posterior probability (Ln P[D]) of each K by averaging the posterior probabilities across the five runs for each K. The number of populations (K) was determined to be five, based on quantitative criteria outlined by Pritchard et al. (2000: maximal value of Ln P[D]) and Evanno et al. (2005: ΔK) (Fig. 1), and consideration of the overall ancestry assignments. The large delta K peak at K = 2 (Fig. 1) probably reflects a larger amount of sub-structure between Wolves and Coyotes than within these species (see Koblmuller et al. 2009), but does not reflect the highest level of population sub-structuring, which we determined to occur at K = 5. Results were consistent between the F-model and I-model of STRUCTURE.
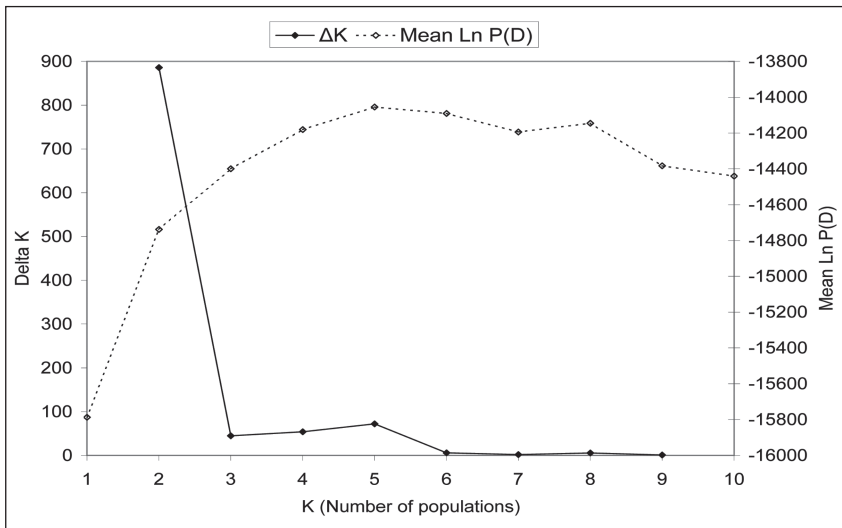

Figure 1. Plots of K determination criteria values, ΔK and Ln P(D), for STRUCTURE analysis of the canid microsatellite genotype data based on 8 loci.

We performed a non-model based factorial correspondence analysis (FCA) on the microsatellite data for individual canids using GENETIX (v4.05; Belkhir et al. 1996–2004). Two factorial components, FC-1 and FC-2, which accounted for 6.84% and 3.66% of the total inertia, respectively, were plotted to visualize the clustering of the eastern Massachusetts samples in relation to the other sample groups.

Nei's standard genetic distances (*D*) (Nei 1972) and pairwise F_{ST} values were calculated in GenAlEx 6.1 (Peakall and Smouse 2006) to estimate genetic differentiation among groups and to determine the most likely origin of founding animals in the study area.

*Parentage and kinship analysis.*Probability of identity (PID) and probability of identity of sibs (PI_{sibs}) (Taberlet and Luikart 1999) were calculated for this dataset in GenAlEx 6.1 (Peakall and Smouse 2006). Field observations and radio-telemetry data suggested probable parent-offspring relationships within some packs. We used mtDNA haplotypes to identify matches between putative mother-offspring. Microsatellite genotypes were used to test the likelihood of suspected parentage with CERVUS 3.0.3 software (Kalinowski et al. 2007). Mothers were excluded if their mtDNA haplotype did not match suspected offspring, and parentage was only assigned when there were no mismatches in the microsatellite data. We did, however, allow for one trio mismatch (among mother-father-offspring groupings) where at least one individual in the comparison was homozygous, if the trio confidence of assignment was at the ≥95% level. The program ML-Relate (Kalinowski et al. 2006) was used to determine maximum-likelihood estimates of pairwise relatedness (*r*) for all individuals (accounting for null alleles) to identify cryptic relationships and pack social structure within the dataset. Accounting for null alleles in kinship analysis reduces the chance of Type II false exclusion errors (e.g., Wagner et al. 2007). Kinship was assigned based on the maximum-likelihood estimates and only if "unrelated" was not consistent with the genetic data at the 0.05 level of significance (except in one case where the assignment of half-siblings was congruent with the other relationships in the pack). In this case, the most likely kinship assignment was accepted even though ML-Relate indicated "unrelated" could also be consistent with the data. Telemetry data (i.e., suspected family units living in the same territory) combined with results from CERVUS and ML-Relate were used to construct pedigrees for 5 packs containing 3–5 individuals per pack.

Results

Genetic analysis

The Massachusetts samples contained only New World *Canis* mtDNA haplotypes (Genbank accessions provided): C1 (*n* = 21, AY267718), C9 (*n* = 26, AY267726), C14 (*n* = 3, AY267731), C19 (*n* = 15, AY267736), and C48 (*n* = 2, FJ687613). Based on the sequence, haplotype C1 is an Eastern Wolf haplotype (Wilson et al. 2000, 2003), and the other four haplotypes are putative Coyote haplotypes (C48 matches la031 and la034 found in Nebraska

Coyotes, C14 matches la033 found in Nebraska coyotes, and C19 matches la006 found in Texas coyotes; see Hailer and Leonard 2008). There did not appear to be a sex bias in the frequency of haplotypes among males and females. In addition, the heavy female Coyote (i.e., "Casper", ID #9804) reported by Way and Proietto (2005) from the town of Barnstable, MA had a C9 mitochondrial DNA haplotype, which clusters with Coyote sequences but has an apparent eastern-specific distribution (i.e., not observed in western coyotes from Texas or Nebraska; Hailer and Leonard 2008), and thus may derive from Eastern Wolves. The microsatellite genotype of this animal clustered with 98.2% assignment to the "eastern Coyote" grouping.

Based on the microsatellite genotypes, five populations were identified by STRUCTURE (Fig. 2): P1 = Massachusetts, Frontenac Axis, Adirondacks, Maine, New York, and New Brunswick; P2 = Texas, Ohio and North Carolina; P3 = Algonquin Park; P4 = northeastern Ontario and Quebec; P5 = Northwest Territories. Based on analyses from previous studies (Grewal et al. 2004; Wheeldon and White 2009; Wilson et al. 2000, 2009) these populations are interpreted as follows: P1 = eastern Coyote (or "coywolf", a name which we suggest better reflects its hybrid origin—see discussion); P2 = western Coyote; P3 = Eastern Wolf; P4 = Gray-Eastern Wolf hybrids; and P5 = Gray Wolves. All of the Massachusetts canids clustered with the eastern Coyote grouping, with very minimal admixture from other populations. The only notable admixture found in Massachusetts canids was for three animals that had a 20–40% assignment probability to the western Coyote population. The FCA plot showed similar groupings to that of STRUCTURE (Fig. 3).

Pairwise comparisons of Nei's genetic distance and F_{ST} values show that Massachusetts canids are most similar to groups of eastern Coyotes from the Adirondacks, New York, Maine, and along the Frontenac Axis in Ontario (Table 1). These data are consistent with hybrid animals originating in Ontario and moving east through Quebec and New York and south into New England, including Cape Cod.

Parentage and kinship analysis

Probability of identity and PID_{sibs} were 1×10^{-6} and 2×10^{-3}, respectively. These values are sufficiently low for individual identification because 1) we
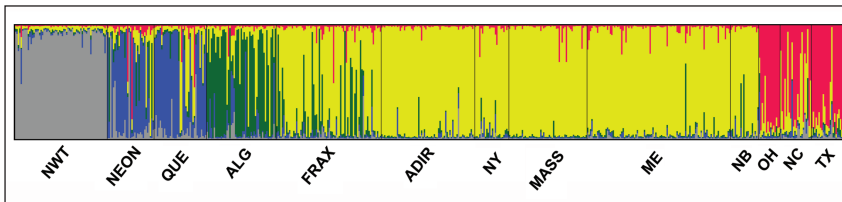

Figure 2. Plot of individual proportional memberships to the K = 5 genetic clusters inferred by STRUCTURE. Each line represents an individual sample and shows the proportional ancestry from each of the five populations, represented by different colors: gray = Gray Wolves, blue = Gray/Eastern Wolf hybrids, green = Eastern Wolf, yellow = eastern Coyote or "coywolf", and red = western Coyote.

were not estimating population size and 2) the mean observed heterozygosity was high ($H_o = 0.64 \pm 0.056$ SE) (Taberlet and Luikart 1999). Maximum likelihood estimates of relatedness accounted for null alleles at 2 loci. We identified parent-offspring relationships in 4 packs: two consisted of an unrelated breeding pair and their offspring, and the other two were mother-offspring groupings (Fig. 4A–D; note: the father was not captured in these groupings but was visually observed traveling with the radio-collared mother). In a 5th pack, a suspected parent-offspring relationship was instead identified as 3 full siblings (Fig. 4E).

Discussion

Genetic analysis

The mtDNA suggest that the genetic diversity of Massachusetts canids originated from both *C. latrans* (Coyotes) and *C. lycaon* (Eastern Wolves), which is consistent with the hypothesis of the hybrid origin of eastern Coyotes. The mtDNA haplotypes found in the Massachusetts canids (except C48) are found in Algonquin Park Eastern Wolves and in eastern Coyotes
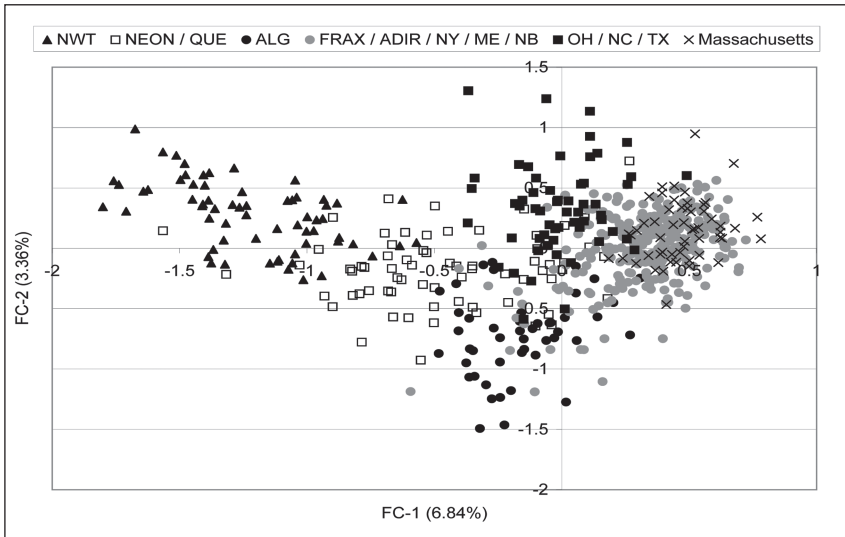

Figure 3. Factorial correspondence analysis of eight microsatellite loci for five *Canis* sample groups. Locality abbreviations are the same as in Table 1.

Table 1. Pairwise comparisons of Nei's genetic distance (*D*) and F_{ST} values between eastern Coyotes in Massachusetts to other putative Coyotes (Adirondacks [ADIR], Maine [ME], New York [NY], Frontenac Axis [FRAX], New Brunswick [NB], Ohio [OH], North Carolina [NC], Texas [TX]), Eastern Wolves (Algonquin [ALG]), Gray Wolves (Northwest Territories [NWT]), and Eastern-Gray Wolf hybrids (Northeast Ontario [NEON], Quebec [QUE]) populations.

	ADIR	ME	NY	FRAX	NB	NC	ALG	TX	OH	NEON	QUE	NWT
F_{ST}	0.012	0.020	0.027	0.033	0.045	0.073	0.125	0.121	0.125	0.134	0.156	0.322
D	0.044	0.059	0.089	0.095	0.123	0.240	0.346	0.366	0.418	0.465	0.499	1.048

south of the Park along the Frontenac Axis, where they are called Tweed Wolves (Grewal et al. 2004; Wilson et al. 2000, 2009). Data from both the mitochondrial haplotypes and the microsatellite loci suggests that Massachusetts canids are *lycaon* x *latrans* hybrids, similar to the Tweed Wolf found in the Frontenac Axis (Wilson et al. 2009). The genetic distance between groups is consistent with the Massachusetts founders originating in southern Ontario and progressing south, down the eastern US and into Massachusetts, rather than from North Carolina or Ohio (Table 1).

The three closely related species of North American *Canis* (western Coyote, Eastern Wolf, and Gray Wolf) do not conform to the biological species concept (Mayr 1942) because they are not reproductively isolated and gene flow occurs between them (Kyle et al. 2006). Although there is no evidence for direct hybridization between Gray Wolves and western Coyotes, the Eastern Wolf mediates gene flow between these two species. This relationship
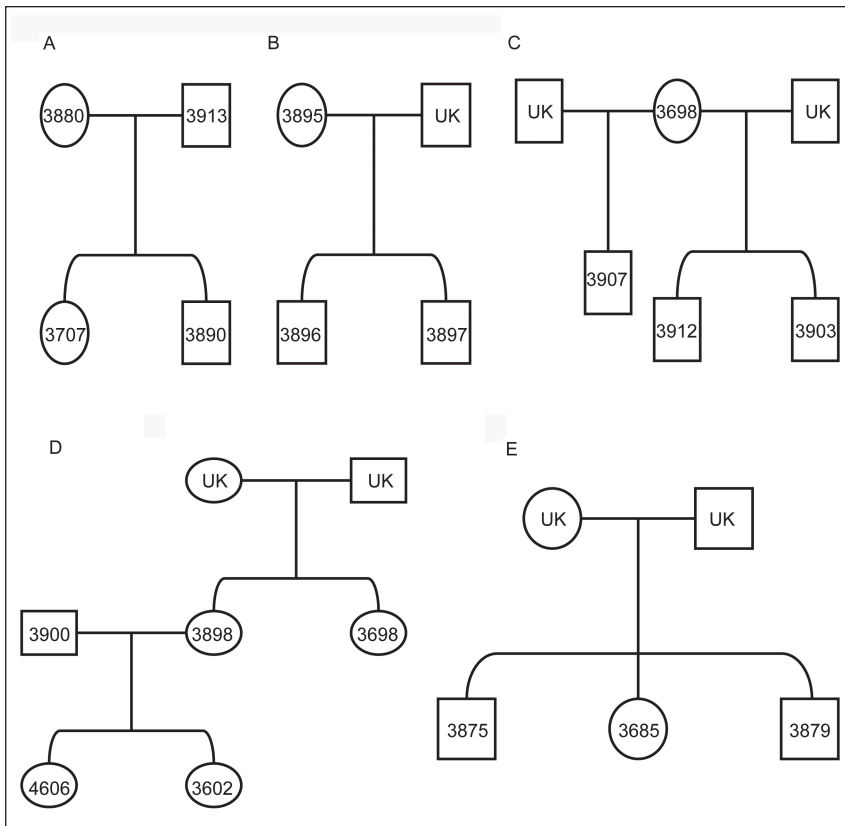

Figure 4. A–E. Pedigrees for five packs of eastern Coyotes from Massachusetts. Circles represent females and squares represent males. All individuals were sampled in this analysis except for unknowns (UK). For example, a radio-collared breeding female may have been sampled along with some of her offspring, while the female's mate may have been uncollared and not sampled, but known to have been present.

is especially apparent in southeastern Ontario where the term "Canis soup" was coined to reflect the mix of eastern Coyotes, Eastern Wolves, Gray Wolves and their hybrids (see Grewal et al. 2004, Sears et al. 2003, Wilson et al. 2009). Microsatellite genotype data presented here provide evidence that the Massachusetts northeastern canids cluster genetically with other eastern Coyote populations and separately from western Coyotes, Eastern Wolves, and Gray Wolves. Because of their morphological and genetic distinctiveness, including from the nearest subspecies of western Coyote, *C. l. thamnos* Jackson, found in the midwest United States (Berg and Chesness 1978, Parker 1995, Way 2007), we suggest that the eastern Coyote be called the "Eastern Coywolf" or just "Coywolf" (*C. latrans* x *lycaon*). This term better reflects the genetic composition of this highly successful canid.

Parentage and kinship analysis

The data suggest that eastern Coyote social groups on Cape Cod and in the Boston area are made up of family groups, similar to those seen in other parts of eastern North America (e.g., Harrison 1992, Patterson and Messier 2001). Offspring typically remain with their parents anywhere from 6 months to about 2 years of age before dispersing to new areas (Harrison et al. 1992); these social units produce a pack of Coyotes. Typically 3–5 adults live together in a territorial pack (Patterson and Messier 2001, Way 2003, Way et al. 2002). Several benefits to social grouping in canids include improved hunting efficiency of large prey (Bekoff et al. 1981, Sand et al. 2006, Schmidt and Mech 1997), defense of territories (Bowen 1981), improved pup survivability (Brainerd et al. 2008), and defense against kleptoparasitism (Vucetich et al. 2004). The relatedness analyses based on microsatellite data suggest that a typical pack consists of related family members, aside from the unrelated breeding pair (Fig. 4). In some cases, we cannot exclude father-son relationships although the maximum likelihood analysis indicates siblings.

Summary of eastern Coyote ecology and behavior

Ecologically, the eastern Coyote behaves as one might predict for a 13.6–18.2 kg (30–40 lb) wild canid. On average, it has a larger home range than most western Coyotes but smaller than wolves, at about 30 km^2 (Mech and Boitani 2003, Patterson and Messier 2001, Way et al. 2002). They also travel long distances daily (16–24 km; Patterson et al. 1999, Way et al. 2004), eat a variety of food including deer, medium-sized prey such as S*ylvilagus* spp. (rabbits), and *Microtus* spp. (voles) (Harrison 1992, Morey et al. 2007, Patterson and Messier 2001), and are social, often living in families of three to five members (Patterson and Messier 2001, Way 2003, Way et al. 2002; note: western Coyotes have also been found to be social where there is abundant prey— see Andelt 1985, Gese et al. 1996). In short, it has ecological and physical characteristics that can be seen on a continuum of Coyote-like to wolf-like. Overall, though, the eastern Coyote seems to occupy an ecological niche that is closer to Coyotes than wolves, which are typically obligate predators of deer (Mech and Peterson 2003, Peterson and Ciucci 2003).

The eastern Coyote, which colonized northeastern North America in the 20th century (Fener et al. 2005, Parker 1995), has a mixture of mitochondrial DNA from Eastern Wolves and naturally colonizing western Coyotes. Although anthropogenic factors such as degradation of original habitat (i.e., conversion of forests into agricultural lands) and wolf-eradication programs facilitated Coyote colonization eastward (Gompper 2002), their expansion and subsequent hybridization with Eastern Wolves was a natural response to environmental changes, making them a naturally evolving member of the faunal community. With changing land-use patterns, hybridization, which is a natural event in nature (Meffe and Carroll 1994), should not be viewed as a negative influence. Rather, it may be enhancing the adaptive potential of both western Coyotes and Eastern Wolves, allowing this emerging new species to more effectively exploit available resources in rapidly changing environments (Kyle et al. 2006). Furthermore, Eastern Wolf genes may be able to persist in regions from which they would otherwise be extirpated (Kyle et al. 2008, Murray and Waits 2007). Kyle et al. (2008) noted that "Coyote/Wolf hybrids are likely harboring Wolf genes that would otherwise be lost due to genetic drift in a small isolated population … and hybridization is moving towards a *Canis* that is better adapted to anthropogenically modified landscapes."

The eastern Coyote has a relatively uniform genetic makeup throughout the Northeast and currently breeds with other eastern Coyotes with minimal influence from other *Canis* types (i.e., western Coyotes or Eastern Wolves; Fig. 2). There is an alternative possibility to widespread hybridization documented in this paper and that involves a small founder effect where the populations of canids in northeastern North America were low due to human exploitation and habitat conversion. This theory postulates that a localized hybridization event occurred between western Coyotes and Eastern Wolves and their offspring subsequently colonized the Northeast. However, given the widespread occurrence of the same mtDNA haplotypes in Eastern Wolf-Coyote hybrids in southern Ontario, and the clear difference of this expansive eastern Coyote population from other *Canis* types, we suggest that widespread hybridization is a more probable explanation than a founder effect.

Scientists, managers, and laypeople should appropriately classify the four canids found in North America belonging to the genus *Canis* as the Western Coyote (*Canis latrans*), Eastern Coyote (or "Coywolf" as we suggest) (*C. latrans* x *lycaon*; east of longitude 80° including New England, New York, New Jersey, Pennsylvania, Ontario, and Quebec), Eastern Wolf (*C. lycaon*, including *C. rufus*), and Gray Wolf (*C. lupus*). A possible fifth group involves Eastern/Gray Wolf hybrids in the Minnesota/Ontario area (see Wheeldon and White 2009). With this "*Canis* soup" of different but closely related species (there is gene flow from *lupus* to *lycaon* [Grewal et al. 2004, Wheeldon and White 2009, Wilson et al. 2009] and *lycaon* to *latrans* [Wilson et al. 2009]), distinct species status for any canid complicates conservation efforts (including *C. lupus*; e.g., Kolenosky 1971, Schmitz and Kolenosky 1985); however, this paper suggests that the eastern Coyote has levels of genetic structure that

are comparable in magnitude to those found between the other species of *Canis* (Figs. 2 and 3). Therefore, it is recommended that future research should extensively sample *Canis* from throughout the US and Canada to better understand the limits of the distributional ranges of the extant *Canis* species in North America and more clearly delimit the areas where hybridization is occurring. The use of noninvasive sampling (e.g., using scat-detecting dogs, rub-posts, snare-posts) could be an efficient method to obtain DNA samples from a wide geographic range (Long et al. 2008)

Implications for wolf recovery into the northeast US

In addition to the eastern Coyote, there have also been a number of wolves (i.e., ca. 30–40 kg, typical wolf-sized animals) that have appeared in the northeastern United States in the past 10 to 20 years (Glowa et al. 2009). These Wolves seem to be either Eastern or Eastern-Gray Wolf hybrids (usually referred to as Gray Wolves, but see Wilson et al. 2009), but have limited Coyote genetic material (see Glowa et al. 2009 and sources within). Current wolf range in southern Canada is within 100 miles of the United States, a distance that wolves could travel in a week or two (Mech and Boitani 2003, Way et al. 2004). Unfortunately, all of these wolves detected in the northeastern US have been found dead before anyone could monitor them (Glowa et al. 2009). Research indicates that habitat exists for wolves in this region (Harrison and Chapin 1997), and as recommended by Kyle et al. (2006), we also suggest that management policies should allow eastern canids to continue to adapt to their changing environment as an efficient means towards establishing a *Canis* population that is able to effectively exploit the available habitat and prey-base. Within this context, issues arise from the difficulty of clearly distinguishing Eastern Wolves from eastern Coyotes based on morphology and their tendency to hybridize, especially where the two are sympatric (e.g., regions of eastern Ontario, Canada).

Because we have a legal obligation to restore a species on the endangered species list to its native range, the difficulty of distinguishing Eastern Wolves from eastern Coyotes/coywolves may have implications for the classification of coywolves under both the Convention on the International Trade of Endangered Species (CITES) and state hunting/trapping legislation, especially considering that Gray Wolves are the only subspecies of Wolf in the northeastern US currently listed under the Endangered Species Act. It may be prudent to allow the eastern Coyote to evolve in response to natural selection without extensive human manipulation (i.e., hunting, trapping), especially given the potentially adaptive hybrid genome inhabiting these regions as observed through the recent emergence of large wolf-like *Canis* in New England (e.g., Way 2007, Way and Proietto 2005).

Most northeastern states allow unlimited killing of eastern Coyotes, yet it does not greatly affect their overall population sizes (see Parker 1995). While western Coyotes, eastern Coyotes, and wolves are all impacted by exploitation in some way (i.e., socially, ecologically, potential for inbreeding, etc.), western and eastern Coyotes are seemingly able to fill the void of

missing individuals more readily (Parker 1995), while wolves are generally more impacted by exploitation (Mech and Boitani 2003). Therefore, better management strategies for the protection of all canids existing in the northeastern US (see Glowa et al. 2009) may result in the natural restoration of a more wolf-like canid in the Northeast. In other words, with current management (i.e., year-long seasons) on eastern Coyotes in most northern US states, wolves have no effective protection if they make it into the northeastern US. While hybridization is a potential problem between eastern Coyotes and any Eastern or Eastern-Gray Wolf that make it into the northeastern US, natural selection may favor a more wolf-like canid if the two are allowed to breed and survive without human killing. It could be argued that Gray Wolves may be a more appropriate source for an active wolf restoration as they likely will not hybridize with eastern Coyotes and may be more ecologically effective predators of larger ungulates like *Alces alces* L. (Moose). However, it will be difficult to find a source of suitable Gray Wolves in the east, as the neighboring wolves in central Ontario and eastern Quebec are Eastern Wolves or Eastern-Gray Wolf hybrids (Wilson et al. 2009).

Editor's note: For the sake of clarity and common understanding, it is journal policy to use widely accepted common names when available in referring to species. The repeated use of the term eastern Coyotes in this manuscript reflects that policy and does not reflect the authors' belief that this sub-population of the species would more accurately be termed Coywolf and their suggestion that Coywolf actually become the standard accepted name .

Literature Cited

Adams, J.R., J.A. Leonard, and L.P. Waits. 2003a. Widespread occurrence of a domestic dog mitochondrial DNA haplotype I in southeastern US Coyotes. Molecular Ecology 12:541–546.

Adams, J.R., B.T. Kelly, and L.P. Waits. 2003b. Using fecal DNA sampling and GIS to monitor hybridization between Red Wolves (*Canis rufus*) and Coyotes (*Canis latrans*). Molecular Ecology 12:2175–2186.

Andelt, W.F. 1985. Behavioral ecology of Coyotes in south Texas. Wildlife Monographs 94:1–45.

Bekoff, M., J. Diamond, and J.B. Mitton. 1981. Life-history patterns and sociality in canids: Body size, reproduction, and behavior. Oecologia 50:386–390.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 1996–2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier, France.

Berg, W.E., and R.A. Chesness. 1978. Ecology of Coyotes in northern Minnesota. Pp. 229–247, *In* M. Bekoff (Ed.). Coyotes: Biology, Behavior, and Management. Academic Press, New York, NY.

Bowen, W.D. 1981. Variation in Coyote social organization: The influence of prey size. Canadian Journal of Zoology 59:639–652.

Brainerd, S.M., H. Andrén, E.E. Bangs, E.H. Bradley, J.A. Fontaine, W. Hall, Y. Iliopoulos, M.D. Jimenez, E.A. Jozwiak, O. Liberg, C.M. Mack, T.J. Meier, C.C. Niemeyer, H.C. Pedersen, H. Sand, R.N. Schultz, D.W. Smith, P. Wabakken, and A.P. Wydeven. 2008. The effects of breeder loss on wolves. Journal of Wildlife Management 71:89–98.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14:2611–2620.

Falush, D., M. Stephens, and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Falush, D., M. Stephens, and J.K. Pritchard. 2007. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. Molecular Ecology Notes 7:574–578.

Fener, H.M., J.R. Ginsberg, E.W. Sanderson, and M.E. Gompper. 2005. Chronology of range expansion of the Coyote, *Canis latrans*, in New York. Canadian Field-Naturalist 119:1–5.

Gese, E.M., R.L. Ruff, and R.L Crabtree. 1996. Social and nutritional factors influencing the dispersal of resident Coyotes. Animal Behaviour 52:1025–1043.

Glowa, J., W.L. Pepperman, C.L. Schadler, J. Butera, and J.G. Way. 2009. Petition submitted to protect wolves and allow wolf recovery in the northeast. Submitted to US Department of Interior on 31 January 2009. 17 pages. Available online at http://easterncoyoteresearch.com/downloads/ESApetition2009final.pdf. Accessed 15 March 2009.

Gompper, M.E. 2002. Top carnivores in the suburbs? Ecological and conservation issues raised by colonization of northeastern North America by coyotes. BioScience 52:185–190.

Grant, P.R., and B.R. Grant. 1997. Hybridization, sexual imprinting, and mate choice. American Naturalist 49:1–28.

Grewal, S.K., P.J. Wilson, T.K. Kung, K. Shami, M.T. Theberge, J.B. Theberge, and B.N. White. 2004. A genetic assessment of the Eastern Wolf (*Canis lycaon*) in Algonquin Provincial Park. Journal of Mammalogy 85:625–632.

Grewal, S.K. 2001. A genetic analysis of the Eastern Timber Wolf. M.Sc. Thesis. McMaster University, Hamilton, ON, Canada. 173 pp.

Hailer, F., and J.A. Leonard. 2008. Hybridization among three native North American *Canis* species in a region of natural sympatry. PloS ONE 3:e3333.

Hall, T.A. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Harrison, D.J. 1992. Social ecology of Coyotes in northeastern North America: Relationships to dispersal, food resources, and human exploitation. Pp. 53–72, *In* A.H. Boer (Ed.). Ecology and Management of the Eastern Coyote. Wildlife Research Unit, University of New Brunswick, Fredericton, NB, Canada.

Harrison, D.J., and T.G. Chapin. 1997. An assessment of potential habitat for Eastern Timber Wolves in the northeastern United States and connectivity with occupied habitat in southeastern Canada. Working Paper Number 7, Wildlife Conservation Society, Bronx, NY.

Hedrick, P.W., R.N. Lee, and D. Garrigan. 2002. Major histocompatibility complex variation in Red Wolves: Evidence for common ancestry with Coyotes and balancing selection. Molecular Ecology 11:1905–1913.

Kalinowski, S.T., M.L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1006.

Kalinowski, S.T., A.P. Wagner, and M.L. Taper. 2006. ML-Relate: A computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes 6:576–579.

Koblmuller, S., M. Nord, R.K. Wayne, and J.A. Leonard. 2009. Origin and status of the Great Lakes Wolf. Molecular Ecology 18(11):2313–2326. Doi: 10.1111/j.1365-294X.2009.04176.x.

Kolenosky, G.B. 1971. Hybridization between wolf and Coyote. Journal of Mammalogy 52:446–449.

Kyle, C.J., A.R. Johnson, B.R. Patterson, P.J. Wilson, K. Shami, S.K. Grewal, and B.N. White. 2006. Genetic nature of Eastern Wolves: Past, present, and future. Conservation Genetics 7:273–287.

Kyle, C.J., A.R. Johnson, B.R. Patterson, P.J. Wilson, and B.N. White. 2008. The conspecific nature of Eastern and Red Wolves: Conservation and management implications. Conservation Genetics 9:699–701.

Larivière, S., and M. Crête. 1993. The size of eastern Coyotes (*Canis latrans*): A comment. Journal of Mammalogy 74:1072–1074.

Lawrence, B., and W.H. Bossert. 1969. Cranial evidence of hybridization in New England *Canis*. Breviora 330:1–13.

Long, R.A., P. MacKay, W.J. Zielinski, J.C. Ray (Eds.). 2008. Noninvasive Survey Methods for Carnivores. Island Press, Washington, DC. 385 pages.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York, NY.

Mech, L.D., and L. Boitani (Eds.). 2003. Wolves: Behavior, Ecology, and Conservation. University of Chicago Press, Chicago, IL.

Mech, L.D., and R.O. Peterson. 2003. Wolf-prey relations. Pp. 131–160, *In* L.D. Mech and L. Boitani (Eds.). Wolves: Behavior, Ecology, and Conservation. University of Chicago Press, Chicago, IL.

Meffe, G.K., and C.R. Carroll. 1994. Principles of Conservation Biology. Sinauer Associates, Sunderland, MA.

Mengel, R.M. 1971. A study of dog-Coyote hybrids and implications concerning hybridization in *Canis*. Journal of Mammalogy 52:316–336.

Morey, P.S., E.M. Gese, and S. Gehrt. 2007. Spatial and temporal variation in the diet of Coyotes in the Chicago metropolitan area. American Midland Naturalist 158:147–161.

Murray, D.L., and L.P. Waits. 2007. Taxonomic status and conservation strategy of the endangered Red Wolf: A response to Kyle et al. (2006). Conservation Genetics 8:1483–1485.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283–291.

Nowak, R.M. 2002. The original status of wolves in eastern North America. Southeastern Naturalist 1:95–130.

Ostrander, E.A., G.F. Sprague, and J. Rine. 1993. Identification and characterization of dinucleotide repeat $(CA)_n$ markers for genetic mapping in dog. Genomics 16:207–213.

Ostrander, E.A., F.A. Mapa, M. Yee, and J. Rine. 1995. One hundred and one new simple sequence repeat-based markers for the canine genome. Mammalian Genome 6:192–195.

Parker, G.R. 1995. Eastern Coyote: The Story of Its Success. Nimbus Publishing, Halifax, NS.

Patterson, B.R., S. Bondrup-Nielsen, and F. Messier. 1999. Activity patterns and daily movements of the eastern Coyote, *Canis latrans*, in Nova Scotia. Canadian Field-Naturalist 113:251–257.

Patterson, B.R., and F. Messier. 2001. Social organization and space use of Coyotes in eastern Canada relative to prey distribution and abundance. Journal of Mammalogy 82:463–477.

Peakall, R., and P.E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Peterson, R.O., and P. Ciucci. 2003. The wolf as a carnivore. Pp. 104–130, *In* L.D. Mech and L. Boitani (Eds.). Wolves: Behavior, Ecology, and Conservation. Chicago University Press, Chicago, IL.

Pilgrim, K.L., D.K. Boyd, and S.H. Forbes. 1998. Testing for wolf-Coyote hybridization in the Rocky Mountains using Mitochondrial DNA. Journal of Wildlife Management 62:683–689.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure from multilocus genotype data. Genetics 155:945–959.

Rambaut, A., and Drummond, A.J. 2009. Tracer [computer program] Available online at http://tree.bio.ed.ac.uk/software/tracer/ 2009. Accessed 15 March 2009.

Sand, H., C. Wikenros, P. Wabakken, and O. Liberg. 2006. Effects of hunting group size, snow depth, and age on the success of wolves hunting Moose. Animal Behaviour 72:781–789.

Schmidt, P.A., and L.D. Mech. 1997. Wolf pack size and food acquisition. American Naturalist 150:513–517.

Schmitz, O.J., and G.B. Kolenosky. 1985. Hybridization between wolf and coyote in captivity. Journal of Mammalogy 66:402–405.

Sears, H.J., J.B. Theberge, M.T. Theberge, I. Thornton, and G.D. Campbell. 2003. Landscape influence on *Canis* morphological and ecological variation in a Coyote-wolf *C. lupus* x *latrans* hybrid zone, southeastern Ontario. Canadian Field-Naturalist 117:589–600.

Shaw, C.N., P.J. Wilson, and B.N. White. 2003. A reliable molecular method of gender determination for mammals. Journal of Mammalogy 84:123–128.

Silver, H., and W.T. Silver. 1969. Growth and behavior of the Coyote-like canid of northern New England with observations on canid hybrids. Wildlife Monographs 17:1–41.

Taberlet, P., and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society 68:41–55.

Theberge, J.B., and M.T. Theberge. 2004. The Wolves of Algonquin Park: A 12-year ecological study. Department of Geography, University of Waterloo, Toronto, ON, Canada.

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

Vucetich, J.A., R.O. Peterson, and T.A. Waite. 2004. Raven scavenging favours group foraging in wolves. Animal Behaviour 67:1117–1126.

Wagner, A.P., S. Creel, L.G. Frank, and S.T. Kalinowski. 2007. Patterns of relatedness and parentage in an asocial, polyandrous Striped Hyena population. Molecular Ecology 16:4356–4369.

Way, J.G. 2003. Description and possible reasons for an abnormally large group size of adult eastern Coyotes observed during summer. Northeastern Naturalist 10:335–342.

Way, J.G. 2007. A comparison of body mass of *Canis latrans* (Coyotes) between eastern and western North America. Northeastern Naturalist 14:111–124.

Way, J.G., and R.L. Proietto. 2005. Record size female Coyote, *Canis latrans*. Canadian Field-Naturalist 119:139–140.

Way, J.G., P.J. Auger, I.M. Ortega, and E.G. Strauss. 2001. Eastern Coyote denning behavior in an anthropogenic environment. Northeast Wildlife 56:18–30.

Way, J.G., I.M. Ortega, and P.J. Auger. 2002. Eastern Coyote home range, territoriality, and sociality on urbanized Cape Cod. Northeast Wildlife 57:1–18.

Way, J.G., I.M. Ortega, and E.G. Strauss. 2004. Movement and activity patterns of eastern Coyotes in a coastal, suburban environment. Northeastern Naturalist 11:237–254.

Wheeldon, T., and B.N. White. 2009. Genetic analysis of historic western Great Lakes region wolf samples reveals early *Canis lupus/lycaon* hybridization. Biology Letters 5:101–104.

Wilson, P.J., S. Grewal, I.D. Lawford, J.N.M. Heal, A.G. Granacki, D. Pennock, J.B. Theberge, M.T. Theberge, D.R. Voigt, W. Waddell, R.E. Chambers, P.C. Paquet, G. Goulet, D. Cluff, and B.N. White. 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., S. Grewal, T. McFadden, R.C. Chambers, and B.N. White. 2003. Mitochondrial DNA extracted from Eastern North American Wolves killed in the 1800s is not of Gray Wolf origin. Canadian Journal of Zoology 81:936–940.

Wilson P.J., S.K. Grewal, F.F. Mallory, and B.N. White. 2009. Genetic characterization of hybrid wolves across Ontario. Journal of Heredity 100:S80–S89. doi:10.1093/jhered/esp034.