BioOne RESEARCH EVOLVED

Coyote colonization of northern Virginia and admixture with Great Lakes wolves

Author(s) :Christine A. Bozarth, Frank Hailer, Larry L. Rockwood, Cody W. Edwards, and Jesús E. Maldonado

Source: Journal of Mammalogy, 92(5):1070-1080. 2011.
Published By: American Society of Mammalogists
DOI: 10.1644/10-MAMM-A-223.1
URL: http://www.bioone.org/doi/full/10.1644/10-MAMM-A-223.1

BioOne (www.bioone.org) is a a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Journal of Mammalogy, 92(5):1070–1080, 2011

Coyote colonization of northern Virginia and admixture with Great Lakes wolves

Christine A. Bozarth,* Frank Hailer, Larry L. Rockwood, Cody W. Edwards, and Jesús E. Maldonado

Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute, Washington, DC 20008, USA (CAB, FH, JEM)
Department of Environmental Science and Policy, George Mason University, Fairfax, VA 22030, USA (CAB, LLR, CWE, JEM)
Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA (JEM)

* *Correspondent: bozarthc@si.edu*

Ecological invasions of generalist species often are facilitated by anthropogenic disturbance. Coyotes (*Canis latrans*) have benefitted from anthropogenic changes to North American ecosystems and have experienced a dramatic range expansion since the early 19th century. The region east of the Mississippi River has been colonized via 2 routes that have converged in the mid-Atlantic region during the past few decades. Coyotes using the northern route of expansion show molecular evidence of admixture with the Great Lakes wolf (GLW). We used noninvasive molecular techniques to detect the geographic origins of the recent coyote colonization of northern Virginia as a representative of the mid-Atlantic region and to detect signatures of admixture with GLWs. Of 455 individual canid scats screened, we sequenced a variable 282-base pair fragment of the mitochondrial control region from 126 coyote scats, assigned individual identities to samples using 6 microsatellite loci, and conducted phylogeographic analyses by comparing our sequences to previously published haplotypes. In 39 individuals identified in our scat surveys we detected 7 mitochondrial DNA haplotypes, all of which have been previously reported in diverse surrounding geographic localities. Phylogeographic analyses indicate multiple sources of colonization of northern Virginia. One common haplotype detected in northern Virginia is of wolf origin, indicating the presence of admixed coyotes and GLWs from the north.

Key words: *Canis latrans*, colonization, control region, coyote, eastern United States, Great Lakes wolf

© 2011 American Society of Mammalogists
DOI: 10.1644/10-MAMM-A-223.1

Ecological invasions can occur due to a combination of biological, environmental, and anthropogenic factors. The arrival of nonnative species often is coupled with human-mediated transport (Lockwood et al. 2007) but also can be facilitated by anthropogenic habitat disturbance (Lockwood et al. 2007). Urbanization has been linked to a decline in native species with specialized niches and an increase in nonnative species with broader niches (McKinney 2006). Anthropogenic alteration of the physical environment, which changes the type and availability of resources, and the presence of competitors are the mechanisms by which this biotic homogenization occurs (Shea and Chesson 2002). Consequently, species that can best take advantage of habitats modified by humans will proliferate.

Members of the genus *Canis* have undergone dramatic range expansions and contractions in North America since the arrival of European colonists in the 17th century. Some taxa, such as the red wolf (*Canis rufus*) and gray wolf (*C. lupus*), have experienced substantial range contractions due to predator control efforts and anthropogenic modification of habitat (Nowak 1979). In contrast, the coyote (*C. latrans*) has rapidly expanded its range from the grasslands and prairies of the American Midwest to most of North and Central America (Bekoff 1977). Since the wave of settlement by European descendants westward across North America in the 1800s, coyotes have colonized west to the Pacific, north to Alaska, east to the Atlantic, and south to Panama (Bekoff 1977).

This proliferation of coyotes is probably the result of 3 anthropogenic influences: eradication of top predators, habitat

modification, and independent translocation events. By the end of the 19th century predator eradication, including bounty programs and widespread poisoning, had decimated nearly all of the gray wolves in the United States and southern Canada (Mech 1970). Gray wolves in North America have been reduced from approximately 2 million individuals (Seton 1929) to approximately 70,000 individuals, with the majority of animals in Canada (Mech and Boitani 2003). This dramatic reduction in the gray wolf population might have released coyotes from the interference competition and the mortality of transient coyotes caused by wolves that previously restricted them to the American Midwest (Berger and Gese 2007; Peterson 1995; Thurber et al. 1992). Release from both predation and competition are implicated in allowing for a biological invasion (Lockwood et al. 2007). Coyotes also are affected by predation and interference competition from cougars (*Puma concolor*), but the 2 species can share food sources and habitat (Koehler and Hornocker 1991).

Concurrent with the decimation of gray wolves across North America, massive anthropogenic habitat change that converted dense forests to agricultural lands opened additional habitat to coyotes (Parker 1995). Coyotes prefer slightly disturbed habitat, as opposed to dense forest, because it increases prey availability (Boisjoly et al. 2010). Coyotes also are adept at exploiting urban habitats, allowing them to populate major cities across the continent (Gibeau 1998; Grinder and Krausman 2001; Howell 1982). These adaptive abilities facilitate the coyote's range expansion across our anthropogenically altered continent. Coyotes also have been translocated for use as pets or hunting animals by private groups, with western coyotes released in the east as early as 1925 (Bekoff 1982; Hilton 1978; Young and Jackson 1951), although the long-term success of these introductions is unknown. Anthropogenic translocations have been documented for several mammal species as early as the Pleistocene (Allen et al. 1989; Flannery and White 1991), but many translocation events likely go undetected because of the inability to distinguish them from other biogeographic events (Grayson 2001).

As coyotes have expanded their range across the eastern United States, hybridization with the significantly more rare red wolves (*C. rufus*) and Great Lakes wolves (GLWs) has become a conservation issue. The range of the red wolf has been drastically reduced by human persecution from much of the southeastern United States to approximately 6,000 km^2 in North Carolina (Adams et al. 2003; Paradiso and Nowak 1972). All members of the current population are direct descendents of a captive breeding program initiated in 1973 when the species received federal protection under the Endangered Species Act (United States Fish and Wildlife Service 2007). Currently, their principal threat to species recovery is hybridization with coyotes, and intensive management operations are ongoing (Kelley 2000; Kelly et al. 1999). The species status of the GLW is currently under intense debate, with recognition of its molecular differentiation from the western gray wolf (*C. lupus*) beginning 20 years ago (Lehman et al. 1991). Differing hypotheses exist regarding the species status of GLW, degree and timing of hybridization–admixture between *C. lupus*, GLWs, and *C. latrans*, and the exact route of the recent colonization by coyotes across the Great Lakes region (Kays et al. 2010; Koblmüller et al. 2009; Kyle et al. 2006; Leonard and Wayne 2008; Nowak 2002; Roy et al. 1994, 1996; Rutledge et al. 2010; Wheeldon et al. 2010; Wheeldon and White 2009; Wilson et al. 2000, 2003, 2009). Regardless of these debates, modern coyote populations in the eastern United States have genetic signatures of admixture with GLWs, with mitochondrial haplotypes that cluster with GLWs found in coyotes in New England and as far south as Pennsylvania (Kays et al. 2010; Koblmüller et al. 2009; Way et al. 2010). Koblmüller et al. (2009) and Way et al. (2010) found mitochondrial DNA (mtDNA) haplotypes of GLW origin in coyotes from New York and Massachusetts. Kays et al. (2010) inferred a region in western New York and Pennsylvania that is a contact zone between 2 advancing fronts of colonizing coyotes, 1 from the West with a high diversity of coyote mtDNA haplotypes and 1 from the Northeast with a lower diversity of coyote mtDNA haplotypes and also GLW haplotypes. From these studies it appears that as coyotes moved through GLW territory, some interbred, and the mitochondrial signatures of that mixing are apparent in northeastern states where coyotes have been studied. To date, coyotes with signatures of admixture with GLWs have not been reported farther south along the East Coast states.

The colonization of North America east of the Mississippi River has occurred over the last 100 years in 2 fronts (Parker 1995), a northern front moving across the Great Lakes region and into the northeastern United States and a southern front moving through the southeastern United States (Fig. 1). The northern front may be further divided into 2 fronts, because coyotes have taken a route north of the Great Lakes and through southeastern Canada and also a route south of the Great Lakes, through Ohio and into the northeastern United States (Kays et al. 2009; Parker 1995). However, these 2 northern fronts have converged in the northeastern United States and can now be considered a single northern front. Using reports of coyote presence from both primary literature and state and federal wildlife agencies, Parker (1995) tracks the expansion of the northern and southern fronts, which seem to converge along the Appalachian Mountains and in the mid-Atlantic region (Delaware, the District of Columbia, Maryland, and Virginia). This region was the last to be colonized by coyotes, as late as 1993 (Parker 1995). At the heart of this region is northern Virginia, a loosely defined area consisting of several counties and independent cities radiating to the south and west of Washington, D.C. This area has a dense human population and has experienced massive population growth in the past decade. Many studies have been made of coyotes throughout much of North America (Gehrt and Prange 2007; Hailer and Leonard 2008; Kamler and Gipson 2000; Kays et al. 2008; Prugh et al. 2005; Riley et al. 2006; Sacks et al. 2008; Way et al. 2010; Wheeldon et al. 2010). However, to date, no peer-reviewed articles focusing on coyotes in the mid-Atlantic states have been published (although coyotes have been a focus of the popular press). In this study we used

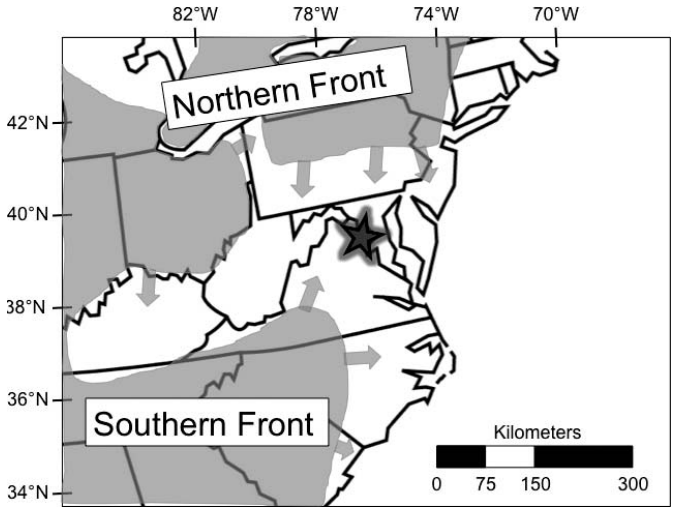

Fig. 1.—Map of the eastern United States showing the northern and southern fronts of coyote expansion converging in the mid-Atlantic region (adapted from Parker 1995). Our study site is marked by a star.

noninvasive molecular technology, a powerful tool to answer ecological questions about elusive species, to obtain sequence data from a variable portion of the mitochondrial control region for coyotes in a newly colonized population in northern Virginia.

Mitochondrial DNA has been used to detect the source of recent colonizations and translocation events for many mammal species (Epps et al. 2010; Kays et al. 2010; Onorato et al. 2004; Van Den Bussche et al. 2009). A recent study by Kays et al. (2010) has correlated haplotype identities and diversity with postulated migration routes to uncover the colonization patterns of coyotes, despite weak phylogeographic structure. Using indicators of colonization patterns across the eastern United States, with 2 fronts apparently merging in the mid-Atlantic region, we sequenced a variable portion of the mitochondrial control region to examine the source of the recent colonization in northern Virginia. Because the mid-Atlantic is near the advancing front of 2 waves of coyote expansion, we hypothesize that this population will show evidence of multiple colonization events from surrounding regions. Additionally, because introgression is known to occur between coyotes and GLWs, we hypothesize that colonization from the northern front will result in the presence of GLW haplotypes in the northern Virginian coyote population.

Materials and Methods

Study site.—This study was conducted in a single area in northern Virginia encompassing 2 land holdings, Marine Corps Base Quantico (MCBQ) and Prince William Forest Park. MCBQ is located in the southern reaches of northern Virginia (Fig. 1), spanning 260 km^2 over 3 counties (Fauquier, Prince William, and Stafford). The base was established in 1917 and was expanded during World War II to its current size. Although MCBQ is an active military reservation, <30% of its holdings are used for strictly military operations. The majority of the land is used for purposes such as forest and wildlife management, potable water production, waste disposal, and outdoor recreation. In the face of burgeoning suburban development surrounding the base, MCBQ and the adjacent Prince William Forest Park (area of ~60 km^2) have become de facto wildlife preserves, hosting a wide variety of plant and animal species. The study site represents a contiguous area of approximately 320 km^2. It is composed of secondary growth deciduous forest, open fields, lakes, streams, and human development. Coyotes were 1st observed on MCBQ in 1997 (T. Stamps, Fish, Wildlife, and Agronomy at Marine Corps Base Quantico, pers. comm.).

Sampling.—We analyzed a total of 124 scats collected at Prince William Forest Park (38°34′N, 78°24′W) during July 2005–September 2008 and 331 scats collected at MCBQ (38°31′N, 77°27′W) during July 2002 and November 2006–October 2008. Scat from Prince William Forest Park (July 2005–September 2008) and from MCBQ in July 2002 was collected opportunistically along roads and trails. Scat from MCBQ obtained from November 2006–October 2008 was collected systematically each month along 500-m transects on roads dispersed throughout the base. Although transects used in all collection sessions at MCBQ were nonrandom due to the restrictions of ongoing military training at the site, randomly generated locations would be ineffective because carnivores often deposit scats on roadways (Macdonald 1980) and humans are inefficient at locating scat in vegetation (Smith et al. 2001, 2003). We also obtained tissue samples from 7 coyotes trapped by hunters at MCBQ.

Amplification of target sequence.—Collected scats were stored in plastic bags in the field and moved to freezers at −20°C within hours of collection. We extracted DNA from scats using QIAGEN QIAamp DNA stool kits (Qiagen Inc., Valencia, California). DNA isolation and amplification (procedures done before polymerase chain reaction) were conducted in a separate room to minimize the risk of contaminating stock DNA with polymerase chain reaction products, and aerosol barrier tips were used for all pre–polymerase chain reaction procedures. Because the scat of sympatric canid species might not be readily identifiable by visual examination (Davison et al. 2002), we conducted species identification of every scat sample following the protocols in Bozarth et al. (2010). This protocol requires the amplification of a short fragment of the control region that is variable in length, identifying each of the potential canid species known to occupy the study area. We reliably differentiated scat deposited by coyote (*C. latrans*), gray fox (*Urocyon cinereoargenteus*), and red fox (*Vulpes vulpes*—Bozarth et al. 2010). We did not detect scat deposited by domestic dog (*C. familiaris*). We sequenced the same 282-base pair (bp) region of the mtDNA control region for all 126 coyote scats detected. We repeated polymerase chain reaction amplification and sequencing for all samples with ambiguous results and amplified and sequenced 7 coyote tissue samples from the study area as positive controls. We used a 20-μl

reaction volume containing 3 μl of template DNA (directly from kit extraction), 0.5 μM of each primer, 1x PCR buffer II, 1.5 mM of $MgCl_2$, 1 unit of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, California), and 0.2 mM of each deoxynucleoside triphosphate. Positive and negative controls were run with each batch of polymerase chain reaction. We amplified DNA in PTC-100 and PTC-200 thermocyclers (MJ Research, Waltham, Massachusetts) using the following program: initial denaturation at 95°C for 11 min; 35 cycles of 30 s at 95°C, 30 s at 58°C, and 2 min at 72°C; and final extension of 72°C for 30 min. Polymerase chain reaction products were cleaned using ExoSAP (USB Corporation, Cleveland, Ohio) and sequenced in both directions using the BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's recommendations. Reactions were purified via centrifugation through Sephadex G-50 columns (Amersham Biosciences, Piscataway, New Jersey). Sequences were run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and aligned by eye in Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan).

Because the sequences used in this study targeted small 282-bp-long fragments ideal for noninvasive samples, and many of the published control region sequences are approximately 385 bp long, we tested the ability of the shorter region to differentiate haplotypes by sequencing a comparably sized fragment for several samples for each of the observed haplotypes to check for diagnostic variable sites outside our 282-bp region. For this we used universal primers L15910 and H16498 (Kocher et al. 1989) in a volume of 20 μl with the same reaction mix described above, except that we used 1 μl of template DNA and 2 mM $MgCl_2$. We used the same procedure as described above, except that we used an annealing temperature of 50°C. Products were cleaned, sequenced, purified, and aligned as above.

Verification of individual identities.—Individual identities of animals detected with scat were verified using up to 6 highly variable tetranucleotide microsatellite loci. Primers FH2001, FH2096, FH2137, FH2140, FH2159, and FH2235 were originally obtained from the canine genome map (Francisco et al. 1996) and adapted and validated for use in population studies of coyotes by Prugh et al. (2005). We used a 25-μl reaction volume containing 5 μl of template DNA (directly from kit extraction), 0.5 μM of each forward FAM, HEX, or TET-labeled forward and unlabeled reverse primer, 1x polymerase chain reaction buffer II, 2 mM of $MgCl_2$, 1 unit of AmpliTaq Gold polymerase (Applied Biosystems), and 0.2 mM of each deoxynucleoside triphosphate. We amplified DNA in PTC-100 and PTC-200 thermocyclers (MJ Research) using the following program: initial denaturation at 95°C for 10 min; 45 cycles of 1 min at 95°C, 1 min at 58°C, and 1 min at 72°C; and final extension of 72°C for 10 min. Polymerase chain reaction products were separated and detected by capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Electropherograms were analyzed using GeneMapper Analysis Software version 4.0 (Applied Biosystems). Each DNA extract was subjected to at least 3 independent polymerase chain reaction amplifications for each locus for allele-size verification. After each scat was successfully typed at all 6 loci the reliability of each observed multilocus score was determined using the program RELIOTYPE (Miller et al. 2002). RELIOTYPE is a program that assesses the reliability of an observed multilocus genotype using a maximum-likelihood approach for minimizing genotyping errors. Because of the low-quality DNA often present in scat samples (Taberlet et al. 1996), not all sequenced samples amplified at all 6 loci. When we found unique alleles at otherwise incomplete multilocus genotypes, we assigned them conservatively as unique individuals.

Comparison to published haplotypes.—To detect the geographical source of this population and to compare indicators of molecular diversity across eastern North America we obtained 156 control region sequences from *Canis* spp. that were published previously in GenBank (Adams et al. 2003; Hailer and Leonard 2008; Kays et al. 2010; Koblmüller et al. 2009; Leonard and Wayne 2008; S. L. Lance, Savannah River Ecology Laboratory, pers. comm.). Because molecular evidence indicates hybridization–admixture between *C. lupus*, GLWs, and *C. latrans* in southeastern Canada, the Great Lakes region, and New England (Kays et al. 2010; Koblmüller et al. 2009; Rutledge et al. 2010; Way et al. 2010), we included haplotypes found in *Canis* from areas surrounding the mid-Atlantic region from animals that were assigned morphologically to any of the 3 taxa. GLW sequences were included specifically to test for the presence of GLW haplotypes in the northern Virginia coyote population, given their recent southward expansion along the North American East Coast (Kays et al. 2010). GenBank sequences were aligned by eye with our coyote sequences in Sequencher 4.8 (Gene Codes Corporation).

Genetic analyses.—We used samples from northern Virginia obtained for this study, and samples from the southeastern United States (Adams et al. 2003; S. L. Lance, Savannah River Ecology Laboratory, pers. comm.), midwestern United States (Hailer and Leonard 2008; Koblmüller et al. 2009), northeastern United States (Kays et al. 2010; Koblmüller et al. 2009), and southeastern Canada (Kays et al. 2010; Leonard and Wayne 2008), to test for sources of the northern Virginia colonization. We used the 2-parameter model of Kimura (Kimura 1981) to construct a neighbor-joining tree (Saitou and Nei 1987) in PAUP* 4.0a109 (Swofford 2002) using the genetic distances between haplotypes and estimating statistical support of branching patterns based on 1,000 bootstrap replicates. For neighbor-joining trees we used a golden jackal (*C. aureus*, accession number AF184048) as an appropriate outgroup because it has been determined previously to be the nearest common ancestor to the *Canis* species included in this study (Wayne et al. 1997).

We generated a statistical parsimony haplotype network using TCS 1.21 (Clement et al. 2000) to visualize potential regional genetic structure. TCS calculates the number of

mutational steps among all pairs of haplotypes and then joins the most similar haplotypes together into a network where their combined probability is >95% (Templeton et al. 1992). We created a network including both our northern Virginia haplotypes and also haplotypes representing potential source populations.

We divided surrounding populations into 6 regions, using published haplotype frequency data and geographic divisions used by other authors (Hailer and Leonard 2008; Kays et al. 2010; Koblmüller et al. 2009; S. L. Lance, Savannah River Ecology Laboratory, pers. comm.). The 6 regions were Texas (TX), Nebraska (NE), South Carolina (SC), Ohio (OH), western Pennsylvania/western New York (PA/NY), and the Northeast (NorE). We used Texas and Nebraska to represent western coyote populations (Hailer and Leonard 2008; Koblmüller et al. 2009). South Carolina represented southeastern coyotes (S. L. Lance, Savannah River Ecology Laboratory, pers. comm.). Northern populations were divided into 3 regions as designated by Kays et al. (2010), with Ohio representing coyotes expanding from the West into the northeastern United States, western Pennsylvania/western New York representing a contact zone between western and eastern coyotes, and the northeastern United States and southeastern Quebec (the Northeast) representing eastern coyotes including admixed coyote–GLW animals. We considered all regions as potential sources of the colonization of northern Virginia.

A newly colonized population is expected to have lower molecular diversity than its source population, with these differences more pronounced when founders are few, the colonization event is recent, and no subsequent gene flow occurs between colony and source (Nei et al. 1975). We calculated nucleotide diversity and haplotype diversity in DnaSP (Liberado and Rozas 2009) for the population in our northern Virginia study area. We compared these values to the same values for studied coyote populations in surrounding areas where these data were available (Hailer and Leonard 2008; Kays et al. 2010; Koblmüller et al. 2009). We also calculated these values for a population of coyotes at the Department of Energy Savannah River Site in South Carolina (S. L. Lance, Savannah River Ecology Laboratory, pers. comm.).

We directly tracked haplotypes across the eastern United States by mapping pie charts of haplotype frequencies for populations in the 6 regions designated above (TX, NE, SC, OH, PA/NY, and NorE) and for the population in our northern Virginia study area. Where haplotype frequency data were not available we mapped only haplotypes that matched ones detected in our study (Adams et al. 2003).

Results

We obtained control region sequences for 89 of the 126 coyote scats and for all 7 coyote tissue samples. Thirty-seven scat samples failed to yield sequence data. The 274 usable sites (<5% missing data) contained 14 transitions, 0 transversions, and 2 insertions–deletions, resulting in 7 haplotypes that have been recovered previously in other *Canis* spp. and have been published in GenBank by other authors (Table 1). However, for the purposes of this paper, our haplotypes will be numbered 1 through 7 (Hap1–Hap7).

We were able to sequence a 385-bp region for representative samples of 5 of our haplotypes to check for diagnostic sites outside of the 282-bp fragment amplified for the remainder of our samples. However, because of problems associated with poor DNA quality characteristic of scat samples, we were unable to amplify the larger fragment for 2 of the haplotypes. These haplotypes were represented by only 1 scat sample each, and DNA degradation could have prevented the amplification of larger fragments. In some cases we were able to amplify multiple scat samples for each haplotype that were determined to be from different individuals based on 6 microsatellite loci. When we compared our short and long sequences to published sequences, with the exception of 2 adjacent transversions that differentiated Hap1 and Hap2, we found no variable sites outside of our short fragment. All 7 haplotypes had diagnostic sites within the short fragment, indicating that our haplotype assignments were reliable and not likely to underestimate the presence of additional haplotypes. Our mtDNA fragment thus appeared appropriate to investigate population structure, molecular diversity, and population origins of North American *Canis* spp.

Verification of individual identities.—We attempted to amplify 6 highly variable microsatellite loci that have been used previously on a coyote population (Prugh et al. 2005) to verify individual identities from the noninvasively captured DNA. Of the 96 samples successfully sequenced (89 scat and 7 tissue), we found 39 individuals among the 65 samples successfully scored. Genotypes were assigned only if they were observed at least 3 times for heterozygotes and 5 times for homozygotes from independent amplifications, as recommended by Taberlet et al. (1996) for noninvasive samples. Additionally, some alleles that did not meet these criteria were assigned using the program RELIOTYPE (Miller et al. 2002), which uses a maximum-likelihood approach to assess the reliability of an observed multilocus genotype. We used 200 bootstrap replicates to estimate the reliability of each allele with ≥95% confidence. Of the 65 samples, 53 were scored at all 6 loci, 7 at 5 loci, 2 at 4, 1 at 3, and 2 at only 1.

Comparison to published haplotypes.—Of the 156 published *Canis* spp. control region sequences found on GenBank, 15 were identical to the haplotypes we detected in northern Virginia (Table 1). Some haplotypes (Hap4, Hap6, and Hap7) detected in northern Virginia were detected in only 1 other locality. Others were detected in multiple localities, often from diverse geographical areas. Hap2 was identical to 2 sequences that have been reported to originate in GLWs (GL1 and GL20).

Population genetic analyses.—We used 149 *Canis* spp. haplotypes obtained from GenBank (we removed 8 of the 15 published haplotypes that were identical to our northern Virginia haplotypes to exclude duplicates; see Table 1). The neighbor-joining tree with bootstrap values revealed a polytomy for coyote–GLW haplotypes (not shown). To simplify the minimum spanning network we included

Table 1.—Published haplotypes that match those observed in *Canis latrans* in northern Virginia. Haplotype name, the locality where it was detected, GenBank accession number, and source publication are shown.

Northern Virginia haplotype	Published haplotype matched	Locality haplotype detected	Accession no.	Source
Hap1	cla25	North Carolina	AY280938	Adams et al. (2003)
	cla29	Northeastern United States	GQ863719	Kays et al. (2010)
	GL16	Quebec	GQ849365	Leonard and Wayne (2008)
Hap2	GL20	Northeastern United States	GQ863717	Kays et al (2010)
	la19	New York and Massachusetts	GQ849373	Koblmüller et al. (2009)
	coy11	South Carolina	EF508170	S. L. Lance, Savannah River Ecology Laboratory, pers. comm.
	GL1	Historic Ontario, Michigan, Wisconsin	GQ849346	Leonard and Wayne (2008)
Hap3	la12	Nebraska	FM209391	Hailer and Leonard (2008)
	coy29	South Carolina	EF508156	S. L. Lance, Savannah River Ecology Laboratory, pers. comm.
Hap4	coy14	South Carolina	EF508166	S. L. Lance, Savannah River Ecology Laboratory, pers. comm.
Hap5	la06	Texas	FM209365	Hailer and Leonard (2008)
	cla28	Northeastern United States	GQ863718	Kays et al. (2010)
	GL11	Quebec	GQ849360	Leonard and Wayne (2008)
Hap6	la15	Nebraska	GQ849371	Koblmüller et al. (2009)
Hap7	cla22	North Carolina	AY280935	Adams et al. (2003)

representatives of each supported (>50%) group within the coyote–GLW clade and all of the haplotypes detected in northern Virginia (Fig. 2). This network showed little structure among coyote haplotypes and many missing haplotypes for this genetically diverse species. Haplotypes of GLW origin (GL2, GL22, and Hap2—Kays et al. 2010; Leonard and Wayne 2008) originated in GLWs and here clustered together in a closely related group. Haplotype la52 is representative of a large clade of coyote haplotypes, including animals from nonhybridizing coyote populations (Adams et al. 2003; Kays et al. 2010; Leonard and Wayne 2008).

Comparisons of molecular diversity between our northern Virginia population and surrounding populations revealed similar levels of nucleotide diversity across eastern North America (Table 2). Haplotype diversity was more variable, with diversity in northern Virginia (0.722) most similar to diversity found in western Pennsylvania/western New York (0.721). In general, a pattern of lower haplotype diversity was seen in the recently colonized eastern states and high haplotype diversity in the long-existing western populations.

All haplotypes detected in northern Virginia had been found previously in populations to the north, south, and west (Fig. 3). Hap1, Hap2, and Hap5 were found in areas both to the north and south of Virginia. Hap3 was found to the south and west of our site, Hap4 and Hap7 were detected only to the south, and Hap6 was observed only to the west.

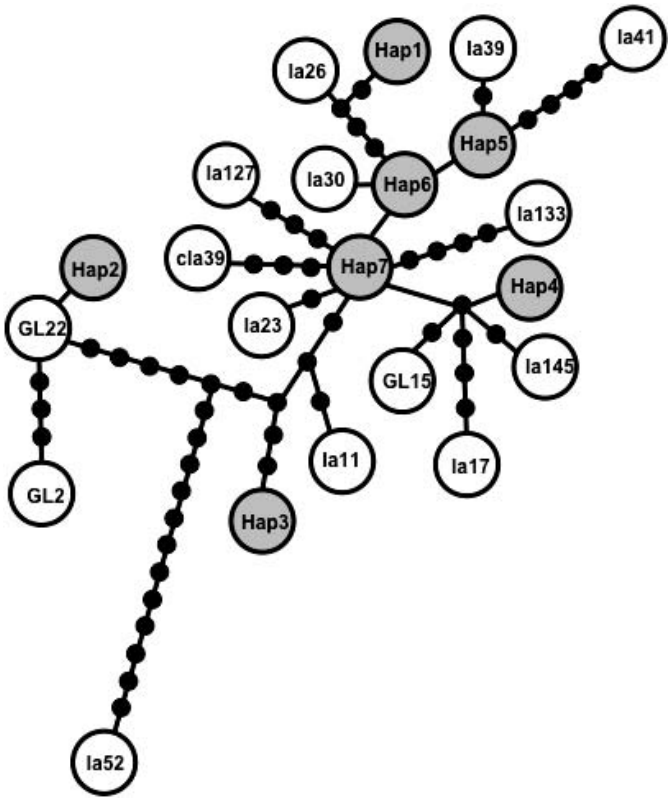

Fig. 2.—Control region minimum-spanning haplotype network based on 385 base pairs (bp) for coyotes collected in northern Virginia for this study (gray shading) and representative haplotypes chosen from each supported (>50%) group within the coyote–Great Lakes wolf clade in the neighbor-joining tree (not shown). Gaps are treated as missing data, not a 5th state. Each node represents a 1-bp change.

Discussion

We used a fast and reliable method to assign haplotypes to coyotes from scat with a short (282-bp) fragment of mtDNA. Smaller fragments are more likely to amplify in noninvasively collected samples because these samples can contain degraded

Table 2.—Genetic diversity in the study population of *Canis latrans* in northern Virginia and in surrounding populations that are potential sources of the colonization. Regions are named with state abbreviations. NoVa = Northern Virginia, NorE = Northeast (defined in Kays et al. 2010), bp = base pairs.

Region	*n*	bp	No. haplotypes	Nucleotide diversity	Haplotype diversity	Source
NoVa	39	366	7	0.016	0.722	This study
NY, MA	48	420–425	7	0.016	0.780	Koblmüller et al. (2009)
OH	30	369	11	0.016	0.844	Kays et al. (2010)
PA/NY	207	369	16	0.015	0.721	Kays et al. (2010)
NorE	450	369	6	0.016	0.664	Kays et al. (2010)
TX	53	393–400	26	0.020	0.949	Hailer and Leonard (2008)
NE	76	393–400	36	0.019	0.967	Hailer and Leonard (2008)
SC	44	445	9	0.015	0.494	S. L. Lance, Savannah River Ecology Laboratory, pers. comm.

DNA (Taberlet et al. 1996). We determined that the short fragment sequenced for most of the scats samples in our study included a highly variable region that contained most of the variable diagnostic sites found in previously reported control region haplotypes, with longer sequences used for comparison in our phylogeographic analysis. Using 6 highly variable microsatellites and extensive verification of allele scores, we identified 39 individuals out of 65 scats successfully scored.

We found that all 7 haplotypes detected in our study also have been observed in coyote populations from diverse geographical localities to the north, south, and west of northern Virginia, consistent with this region being the terminus of colonization in the continental United States. Our neighbor-joining tree and haplotype network indicate little phylogeographic structure in coyotes. This result is in concordance with mtDNA analysis of coyotes across North

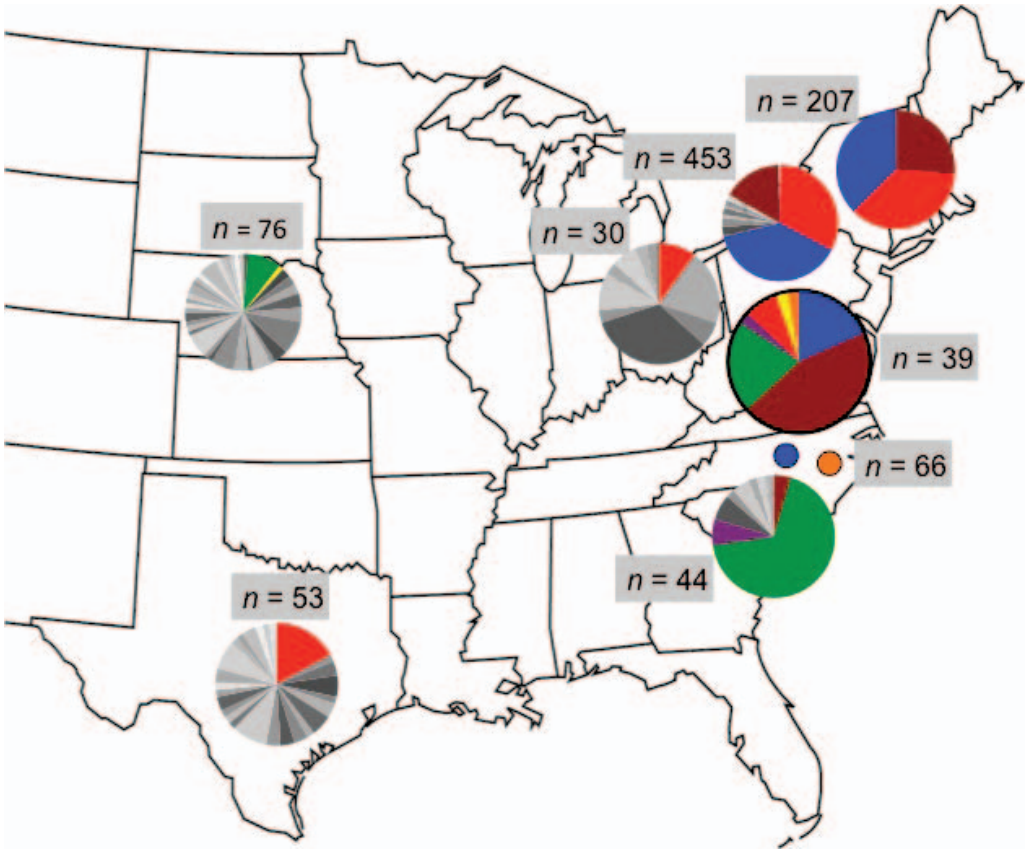

Fig. 3.—Map of coyote haplotype frequencies in northern Virginia (this study; highlighted by black border around pie chart); Texas and Nebraska (Hailer and Leonard 2008; Koblmüller et al. 2009); South Carolina (S. L. Lance, Savannah River Ecology Laboratory, pers. comm.); and Ohio, western Pennsylvania/western New York, and the Northeast (Kays et al. 2010). Colored pie wedges represent haplotypes also found in northern Virginia. Hap1 is blue, Hap2 is brown, Hap3 is green, Hap4 is purple, Hap5 is red, Hap6 is yellow, and Hap7 is orange. Where haplotype frequency data were not available, small colored circles indicate the presence of haplotypes also occurring in northern Virginia. Sample sizes (*n*) are shown for all populations.

America by Lehman and Wayne (1991), which also showed weak phylogeographic partitioning, with similar haplotypes found in animals from such distant localities as California and Nebraska. Weak phylogeographic structure of mtDNA has been found in other canids and has been attributed to the high dispersal abilities of these animals (Dalen et al. 2005; Lehman and Wayne 1991; Vilà et al. 1999). The haplotype network showing the relationship of published haplotypes from surrounding regions places our northern Virginia haplotypes throughout the network. This suggests that northern Virginia was colonized by coyotes from diverse geographical sources most likely multiple times rather than a single colonization by closely related matrilines.

The haplotype diversity of the northern Virginia coyote population most closely resembles that of the western Pennsylvania/western New York population. The western Pennsylvania/western New York population was shown by Kays et al. (2010) to be a contact zone where coyotes expanding from the West through Ohio have interbred with coyotes expanding southward along the East Coast from the Northeast. Examination of our data shows that this contact zone is located as far south as northern Virginia. This region is thus a contact zone between coyotes expanding from the north, south, and west. This lower haplotype diversity along the newly colonized East Coast stands in contrast to populations in the West (e.g., Texas and Nebraska) where coyote populations have been long established and exhibit higher diversity (Hailer and Leonard 2008). This pattern of reduced genetic diversity in regions of recent colonization is typical of a leptokurtic pattern of dispersal (Hewitt 1996; Ibrahim et al. 1996) and has been observed for many mammalian species that have experienced range expansions (e.g., eastern woodrat [*Neotoma floridana*—Hayes and Harrison 1992], northern flying squirrel [*Glaucomys sabrinus*—Arbogast 1999], and *V. vulpes* [Aubry et al. 2009]). The low haplotype diversity observed in South Carolina thus could be a result of a recent founder effect, and future influx of additional haplotypes could occur if immigration continues.

Despite weak large-scale phylogeographic structure in coyotes, as shown in other studies (Lehman and Wayne 1991) and also found in this study, the pattern of low haplotype diversity in the Northeast, with higher diversity to the south and west, is evident. Examination of other occurrences of haplotypes found in northern Virginia indicates multiple sources of colonization. Although sampling to the west of the mid-Atlantic region is incomplete, direct tracking of haplotypes along known coyote colonization routes provides strong evidence for sources of colonization. Hap1, Hap2, and Hap5 are the predominant haplotypes in the Northeast and western Pennsylvania/western New York populations, and their spread further south into Virginia, North Carolina, and South Carolina is consistent with their previously documented southward expansion along the East Coast (Kays et al. 2010). Therefore, although those 3 haplotypes also have been detected to the south and west of our study site, they most likely colonized northern Virginia from the north. Because Hap3, Hap4, Hap6, and Hap7 were not detected in the well-sampled area to the north, they presumably colonized northern Virginia from the west or the south. This direct evidence of colonization from multiple surrounding regions is in concordance with the reported colonization route (Parker 1995) of coyotes moving eastward along northern and southern fronts of expansion and terminating in the mid-Atlantic. In addition, the success of an ecological invasion is reliant on the number of colonists and the number of colonization events (Kolbe et al. 2004; Lockwood et al. 2007). In this case, although the number of colonists is unknown, evidence exists for multiple colonization events and at least 7 maternal founding lineages.

We found a haplotype (Hap2) that is identical to 2 reported GLW sequences, GL1 and GL20. Haplotype GL1 was found in historic (~100 years old) specimens from Ontario, Michigan, and Wisconsin (Leonard and Wayne 2008). These specimens were collected before coyote colonization in the region and, as such, are not the products of recent admixture. Haplotype GL20 is identical to GL1, except that GL20 is a shorter fragment and probably indicates that haplotype GL1 is extant and currently expanding southward along the East Coast (Leonard and Wayne 2008). This GLW haplotype is common in coyotes in the northeastern United States and southeastern Quebec (Kays et al. 2010) and was found in 15 of 48 coyotes sampled in New York and Massachusetts (la19—Koblmüller et al. 2009). Not only do animals possessing a GLW mtDNA haplotype show genetic signatures of admixture with GLWs, but these animals also possess craniodental characteristics more similar to wolves than coyotes (Kays et al. 2010), demonstrating the ecological significance of this admixture event.

In this study we showed that noninvasive molecular technology can be an effective tool to detect the origins of a recent colonization of an elusive carnivore despite weak phylogeographic structure across its range. We also have detected a haplotype (Hap2) that was described only recently in coyotes having signatures of admixture with the GLW in the northeastern United States and southeastern Canada. We found this haplotype to be common in the northern Virginia coyote population. This haplotype was found in 2 coyotes from South Carolina (S. L. Lance, Savannah River Ecology Laboratory, pers. comm.), extending the reported range of admixed coyote–GLW animals even further south. Notably, this southward expansion brings admixed coyote–GLW animals into the range of the red wolf reintroduction program in North Carolina. Currently, the exact taxonomic relationship between coyotes, GLWs, and red wolves is under debate in the literature, and this observed expansion of admixed coyote–GLW individuals into the range of the critically endangered red wolf might further complicate the issue. Regardless, it is clear that the coyote has been able to expand dramatically through the ranges of other *Canis* spp. despite anthropogenic persecution and probably will continue to dominate the eastern United States as its principal mammalian predator.

Acknowledgments

Financial and logistical assistance was provided by Naval Facilities Engineering Command Washington Cooperative Agreement N40080-08-LTC-0001 for Coyote Population Study at MCBQ, the Smithsonian Conservation Biology Institute Center for Conservation and Evolutionary Genetics, and the Smithsonian Institution National Museum of Natural History. We thank T. Stamps, C. Himmelberger, K. Robinson, N. Rotzel, and L. Lakeman for samples, assistance, and guidance. We also thank S. Lance and J. Leonard for providing haplotype data from their studies.

Literature Cited

Adams, J. R., B. T. Kelly, and L. P. Waits. 2003. Using faecal DNA sampling and GIS to monitor hybridization between red wolves (*Canis rufus*) and coyotes (*Canis latrans*). Molecular Ecology 12:2175–2186.

Allen, J., C. Gosden, and J. P. White. 1989. Human Pleistocene adaptations in the tropical island Pacific: recent evidence from New Ireland, a greater Australian outlier. Antiquity 63: 548–561.

Arbogast, B. S. 1999. Mitochondrial phylogeography of the New World flying squirrels (*Glaucomys*): implications for Pleistocene biogeography. Journal of Mammalogy 80:142–155.

Aubry, K. B., M. J. Statham, B. N. Sacks, J. D. Perrine, and S. M. Wisely. 2009. Phylogeography of the North American red fox: vicariance in Pleistocene forest refugia. Molecular Ecology 18:2668–2686.

Bekoff, M. 1977. *Canis latrans*. Mammalian Species 79:1–9.

Bekoff, M. 1982. Coyote. Pp. 447–459 in Wild mammals of North America: biology, management, and economics (J. A. Chapman and G. A. Feldhamer, eds.). Johns Hopkins University, Baltimore, Maryland.

Berger, K. M., and E. M. Gese. 2007. Does interference competition with wolves limit the distribution and abundance of coyotes? Journal of Animal Ecology 76:1075–1085.

Boisjoly, D., J. Ouellet, and C. Rehaume. 2010. Coyote habitat selection and management implications for the Gaspesie caribou. Journal of Wildlife Management 74:3–11.

Bozarth, C. A., et al. 2010. An efficient noninvasive method for discriminating among faeces of sympatric North American canids. Conservation Genetics Resources 2:173–175.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

Dalen, L., et al. 2005. Population history and genetic structure of a circumpolar species: the arctic fox. Biological Journal of the Linnean Society 84:79–89.

Davison, A., J. D. S. Birks, R. C. Brookes, T. C. Braithwaite, and J. E. Messenger. 2002. On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. Journal of Zoology (London) 257:141–143.

Epps, C. W., J. D. Wehausen, P. J. Palsboll, and D. R. McCullough. 2010. Using genetic tools to track desert bighorn sheep colonizations. Journal of Wildlife Management 74:522–531.

Flannery, T. F., and J. P. White. 1991. Animal translocation. National Geographic Research and Exploration 7:96–113.

Francisco, L. V., A. A. Langston, C. S. Mellersh, C. L. Neal, and E. A. Ostrander. 1996. A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. Mammalian Genome 7:359–362.

Gehrt, S. D., and S. Prange. 2007. Interference competition between coyotes and raccoons: a test of the mesopredator release hypothesis. Behavioral Ecology 18:204–214.

Gibeau, M. L. 1998. Use of urban habitats by coyotes in the vicinity of Banff Alberta. Urban Ecosystems 2:129–139.

Grayson, D. K. 2001. The archaeological record of human impacts on animal populations. Journal of World Prehistory 15:1–68.

Grinder, M. I., and P. R. Krausman. 2001. Home range, habitat use, and nocturnal activity of coyotes in an urban environment. Journal of Wildlife Management 65:887–898.

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.

Hayes, J. P., and R. G. Harrison. 1992. Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the eastern United States. Systematic Biology 41: 331–344.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58:247–276.

Hilton, H. 1978. Systematics and ecology of the eastern coyote. Pp. 209–228 in Coyotes: biology, behavior, and management (M. Bekoff, ed.). Academic Press, New York.

Howell, R. G. 1982. The urban coyote problem in Los Angeles County. Pp. 20–23 in Proceedings of the Tenth Vertebrate Pest Conference, University of Nebraska, Lincoln.

Ibrahim, K. M., R. A. Nichols, and G. M. Hewitt. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77:282–291.

Kamler, J. F., and P. S. Gipson. 2000. Space and habitat use by resident and transient coyotes. Canadian Journal of Zoology 78:2106–2111.

Kays, R. W., A. Curtis, and J. J. Kirchman. 2009. Rapid adaptive evolution of northeastern coyotes via hybridization with wolves. Biology Letters 5:1–5.

Kays, R. W., A. Curtis, and J. J. Kirchman. 2010. Reply to Wheeldon et al. 'Colonization history and ancestry of northeastern coyotes.' Biology Letters 6:248–249.

Kays, R. W., M. E. Gompper, and J. C. Ray. 2008. Landscape ecology of eastern coyotes based on large-scale estimates of abundance. Ecological Applications 18:1014–1027.

Kelly, B. T. 2000. Red wolf recovery program adaptive work plan—FY00 to FY02. United States Fish and Wildlife Service, Manteo, North Carolina, pp. 1–15.

Kelly, B. T., P. S. Miller, and U. S. Seal (eds.). 1999. Population and habitat viability assessment workshop for the red wolf (*Canis rufus*). Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, Minnesota.

Kimura, M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. Proceedings of the National Academy of Sciences 78:454–458.

Koblmüller, S., M. Nord, R. K. Wayne, and J. A. Leonard. 2009. Origin and status of the Great Lakes wolf. Molecular Ecology 18:2313–2326.

Kocher, T. D., et al. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences 86:6196–6200.

Koehler, G. M., and M. G. Hornocker. 1991. Seasonal resource use among mountain lions, bobcats, and coyotes. Journal of Mammalogy 72:391–396.

KOLBE, J. J., R. E. GLOR, L. RODRÍGUEZ SCHETTINO, A. C. LARA, A. LARSON, AND J. B. LOSOS. 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181.

KYLE, C. J., ET AL. 2006. Genetic nature of eastern wolves: past, present and future. Conservation Genetics 7:273–287.

LEHMAN, N., ET AL. 1991. Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. Evolution 45:104–119.

LEHMAN, N., AND R. K. WAYNE. 1991. Analysis of coyote mitochondrial DNA genotype frequencies: estimation of the effective number of alleles. Genetics 128:405–416.

LEONARD, J. A., AND R. K. WAYNE. 2008. Native Great Lakes wolves were not restored. Biology Letters 4:95–98.

LIBERADO, P., AND J. ROZAS. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

LOCKWOOD, J. L., M. F. HOOPES, AND M. P. MARCHETTI. 2007. Invasion ecology. Blackwell Publishing, Malden, Massachusetts.

MACDONALD, D. W. 1980. Patterns of scent marking with urine and faeces amongst carnivore communities. Symposia of the Zoological Society of London 45:107–139.

MCKINNEY, M. L. 2006. Urbanization as a major cause of biotic homogenization. Biological Conservation 127:247–260.

MECH, L. D. 1970. The wolf: ecology and behavior of an endangered species. University of Minnesota Press, Minneapolis.

MECH, L. D., AND L. BOITANI. 2003. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, Illinois.

MILLER, C. R., P. JOYCE, AND L. P. WAITS. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160:357–366.

NEI, M., T. MARUYAMA, AND R. CHAKRABORTY. 1975. Bottleneck effect and genetic variability in populations. Evolution 29:1–10.

NOWAK, R. M. 1979. North American Quaternary *Canis*. Museum of Natural History, University of Kansas, Lawrence.

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

ONORATO, D. P., E. C. HELLGREN, R. A. VAN DEN BUSSCHE, AND D. L. DOAN-CRIDER. 2004. Phylogeographic patterns within a metapopulation of black bears (*Ursus americanus*) in the American Southwest. Journal of Mammalogy 85:140–147.

PARADISO, J. L., AND R. M. NOWAK. 1972. *Canis rufus*. Mammalian Species 22:1–4.

PARKER, G. 1995. Eastern coyote: the story of its success. Nimbus Publishing, Halifax, Nova Scotia, Canada.

PETERSON, R. O. 1995. Wolves as interspecific competitors in canid ecology. Pp. 315–324 in Ecology and conservation of wolves in a changing world (L. N. Carbyn, S. H. Fritts, and D. R. Seip, eds.). Circumpolar Press, Edmonton, Alberta, Canada.

PRUGH, L. R., C. E. RITLAND, S. M. ARTHUR, AND C. J. KREBS. 2005. Monitoring coyote population dynamics by genotyping faeces. Molecular Ecology 14:1585–1596.

RILEY, S. P. D., ET AL. 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. Molecular Ecology 15:1733–1741.

ROY, M. S., E. GEFFEN, D. SMITH, E. A. OSTRANDER, AND R. K. WAYNE. 1994. Pattern of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution 11:553–570.

ROY, M. S., E. GEFFEN, D. SMITH, AND R. K. WAYNE. 1996. Molecular genetics of pre-1940 red wolves. Conservation Biology 10:1413–1424.

RUTLEDGE, L. Y., C. J. GARROWAY, K. M. LOVELESS, AND B. R. PATTERSON. 2010. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. Heredity 10:1–12.

SACKS, B. N., D. L. BANNASCH, B. B. CHOMEL, AND H. B. ERNEST. 2008. Coyotes demonstrate how habitat specialization by individuals of a generalist species can diversify populations in a heterogenous ecoregion. Molecular Biology and Evolution 25:1384–1394.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425.

SETON, E.T. 1929. Lives of game animals. Doubleday, Doran & Co., Inc., New York.

SHEA, K., AND P. CHESSON. 2002. Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution 17:170–176.

SMITH, D. A., K. RALLS, B. DAVENPORT, B. ADAMS, AND J. E. MALDONADO. 2001. Canine assistants for conservationists. Science 291:435.

SMITH, D. A., ET AL. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Animal Conservation 6:339–346.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

TABERLET, P., ET AL. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24:3189–3194.

TEMPLETON, A. R., K. A. CRANDALL, AND C. F. SING. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. Genetics 132:619–633.

THURBER, J. M., R. O. PETERSON, J. D. WOOLINGTON, AND J. A. VUCETICH. 1992. Coyote coexistence with wolves on the Kenai Peninsula, Alaska. Canadian Journal of Zoology 70:2494–2498.

UNITED STATES FISH AND WILDLIFE SERVICE. 2007. Red wolf (*Canis rufus*) five year status review: summary and evaluation. Alligator River National Wildlife Refuge, Manteo, North Carolina.

VAN DEN BUSSCHE, R. A., ET AL. 2009. Mitochondrial DNA phylogeography of black bears (*Ursus americanus*) in central and southern North America: conservation implications. Journal of Mammalogy 90:1075–1082.

VILÀ, C., ET AL. 1999. Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. Molecular Ecology 8:2089–2103.

WAY, J. G., L. RUTLEDGE, T. WHEELDON, AND B. N. WHITE. 2010. Genetic characterization of eastern ''coyotes'' in eastern Massachusetts. Northeastern Naturalist 17:189–204.

WAYNE, R. K., E. GEFFEN, D. J. GIRMAN, K. P. KOEPFLI, L. M. LAU, AND C. R. MARSHALL. 1997. Molecular systematics of the Canidae. Systematic Biology 46:622–653.

WHEELDON, T., B. R. PATTERSON, AND B. N. WHITE. 2010. Colonization history and ancestry of northeastern coyotes. Biology Letters 6:246–247.

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., ET AL. 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. K. GREWAL, F. F. MALLORY, AND B. N. WHITE. 2009. Genetic characterization of hybrid wolves across Ontario. Journal of Heredity 100:S80–S89.

WILSON, P. J., S. GREWAL, T. MCFADDEN, R. E. 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.

YOUNG, S. P., AND H. H. T. JACKSON. 1951. The clever coyote. University of Nebraska Press, Lincoln.

Submitted 29 June 2010. Accepted 23 April 2011.

Associate Editor was Samantha Wisely.