Phylogeography of the gray fox (*Urocyon cinereoargenteus*) in the eastern United States

Author(s): Christine A. Bozarth, Stacey L. Lance, David J. Civitello, Julie L. Glenn, and Jesús E. Maldonado

Source: Journal of Mammalogy, 92(2):283-294. 2011.
Published By: American Society of Mammalogists
DOI: http://dx.doi.org/10.1644/10-MAMM-A-141.1
URL: http://www.bioone.org/doi/full/10.1644/10-MAMM-A-141.1

Journal of Mammalogy, 92(2):283–294, 2011

Phylogeography of the gray fox (*Urocyon cinereoargenteus*) in the eastern United States

Christine A. Bozarth,* Stacey L. Lance, David J. Civitello, Julie L. Glenn, and Jesús E. Maldonado

Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute, Washington, DC 20008, USA (CAB, JEM)
Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013 (JEM)
Department of Environmental Science and Public Policy, George Mason University, Fairfax, VA 22030, USA
Department of Biology, Colby College, Waterville, ME 04901, USA (SLL, DJC)
Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA (SLL)
Department of Biology, Indiana University, Bloomington, IN 47401, USA (DJC)
Peromyscus Genetic Stock Center, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA (JLG)

* *Correspondent: bozarthc@si.edu*

Molecular data have been used to show northward post-Pleistocene range expansions from a refugium in the southeastern United States for several mammal species. Fossil and historical records indicate that gray foxes (*Urocyon cinereoargenteus*) were not present in the northeastern United States until well after the Pleistocene (ca. 900). To test the hypothesis that gray foxes experienced a post-Pleistocene range expansion we conducted a phylogeographic analysis of gray foxes from across the eastern United States. We sequenced a variable portion of the mitochondrial control region (411 base pairs) from 229 gray fox tissue samples from 15 states, representing the range of all 3 East Coast subspecies. Phylogeographic analyses indicated no clear pattern of genetic structuring of gray fox haplotypes across most of the eastern United States. However, when haplotype frequencies were subdivided into a northeastern and a southern region, we detected a strong signal of differentiation between the Northeast and the rest of the eastern United States. Indicators of molecular diversity and tests for demographic expansion confirmed this division and suggested a very recent expansion of gray foxes into the northeastern states. Our results support the hypothesis that gray foxes 1st colonized the Northeast during a historical period of hemisphere-wide warming, which coincided with the range expansion of deciduous forest. We present the 1st study that analyzes the phylogeographic patterns of the gray fox in the eastern United States.

Key words: d-loop, eastern United States, gray fox, phylogeography, Pleistocene, *Urocyon*

DOI: 10.1644/10-MAMM-A-141.1

Both molecular and fossil data have been used to document the range shift of animal species from Pleistocene refugia to modern distributions. Genetic data have been used to show Holocene range expansions from low-latitude refugia and the resultant genetic structure for several mammal species in eastern North America, including flying squirrels, short-tailed shrews, and woodrats (Arbogast 1999, 2007; Brant and Orti 2003; Ellsworth et al. 1994; Hayes and Harrison 1992; Petersen and Stewart 2006).

The gray fox (*Urocyon cinereoargenteus*) is a wide-ranging mammal that occupies wooded, brushy, and rocky habitats and is strongly associated with deciduous forest (Fritzell and Haroldson 1982). It ranges from southern Canada throughout North America and montane habitats in central and northern South America (Fuller and Cypher 2004). This range includes areas that have been colonized over the last century, including notable expansions into the northeastern states, eastern Canada, and through the plains states of the Midwest—for a complete list of states and provinces see Fritzell and Haroldson (1982). Sixteen subspecies have been described across the distribution range of the gray fox (Wilson and Reeder 2005). Despite their widespread distribution remarkably little examination of their population genetics has been undertaken (but see Weston Glenn et al. 2009).

The gray fox is 1 of only 2 extant members of the genus *Urocyon* (the other is *U. littoralis*, the Channel Island fox). *Urocyon* evolved in North America and has been present since the end of the Hemphillian age (5 million years ago [mya]) at the start of the Pliocene (Kurtén and Anderson 1980). *U. progressus*, presumed to be an ancestral form of *U. cinereoargenteus*, is the earliest known member of the genus and lived throughout the Blancan age (5 mya–2 mya). Evidence of another extinct species, *U. atwaterensis* (possibly a transitional form), has been dated to the late Blancan age. Paleontological evidence of *U. cinereoargenteus* begins in the early Irvingtonian age (2 mya) with fossils found in Arkansas, Florida, Maryland, and Pennsylvania. In the mid-Atlantic ample evidence of gray foxes exists in the archaeological record (Kurtén and Anderson 1980). John Smith noted their presence along the James River in 1612, British settlers hunted them for sport in Maryland in 1650, and William Byrd recorded them in Virginia in 1730 (Linzey 1998). In the northeastern states fossil evidence of gray foxes is recent and sparse, with fossils of *U. cinereoargenteus* found on Martha's Vineyard, Massachusetts, dating to 400–1,100 years ago (Huntington 1959) and fossils found in southern Connecticut dating to 200–350 years ago (Waters 1965). Gray foxes were observed to be rare by the pilgrims in Massachusetts about 1635, who noted the presence of ''two or three kinds of fox, one a great yellow Fox, another Grey, who will climb up into trees'' (Keay 1901:540). The yellow fox refers to the subspecies of native red fox (*Vulpes vulpes rubricosa*) whose range encompassed northern New England before European settlement, just barely extending to the south into Massachusetts (Churcher 1959; Kamler and Ballard 2002). By the mid-18th century gray foxes had disappeared from the Northeast, possibly due to competition with the introduced red fox from Europe (Churcher 1959; but see Trapp and Halberg 1975). Since then gray foxes have reappeared in the northeastern states and expanded their range even further north (Fritzell and Haroldson 1982; Trapp and Halberg 1975).

Along the East Coast of North America are 3 recognized subspecies of gray fox differentiated only by size and pelage color: *U. c. borealis* (Merriam 1903) in the Northeast, *U. c. cinereoargenteus* (Schreber 1775) in the mid-Atlantic states east of the Mississippi and the Southeast north of Georgia, and *U. c. floridanus* (Rhoads 1895) in the Gulf Coast states (Fig. 1). Although division into subspecies is common practice, many furbearers do not display regional genetic structure in concordance with subspecies designations (Helgen et al. 2008; Lance et al. 2003; Lehman and Wayne 1991). This may be due to anthropogenic translocations or the extensive dispersal abilities of many of these mammals. In addition, pelage color, upon which gray fox subspecies designations are based, has been shown to be ecophenotypically plastic for mammals (Patton and Brylski 1987).

In this study we sequenced a 411-base pair (bp) fragment of the control region of the mitochondrial DNA (mtDNA) to examine the phylogeography of gray foxes in the eastern United States. mtDNA in mammals mutates 5- to 10-fold faster than single-copy nuclear genes, and variable blocks of the control region mutate 4- to 5-fold faster than the remainder of the mtDNA (Taberlet 1996). Because of this rapid mutation rate, the control region has been used in many phylogeographic studies to detect genetic structure and signatures of demographic change in North American mammals (Aubry et al. 2009; Helgen et al. 2008; Lance et al. 2003; Petersen and Stewart 2006; Rowe et al. 2004, 2006; Vilà et al. 1999; Wisely et al. 2008). The control region is an ideal marker for studying gray foxes because they can show subtle genetic structure and might have experienced very recent demographic changes. We examined a hypervariable fragment of the mitochondrial control region for gray foxes from throughout the range of all 3 East Coast subspecies to test the following 2 phylogeographic hypotheses. First, gray foxes experienced a demographic expansion from a southern refugium much more recently than other post-Pleistocene colonizers (<10,000 years ago). Therefore, we expect reduced haplotype and nucleotide diversity as sampling moves away from the southern source and also expect signatures of demographic expansion in the South. Second, modern gray foxes show little genetic structure across the eastern United States, as has been shown for other mammal species, despite morphological differentiation that characterizes each subspecies.

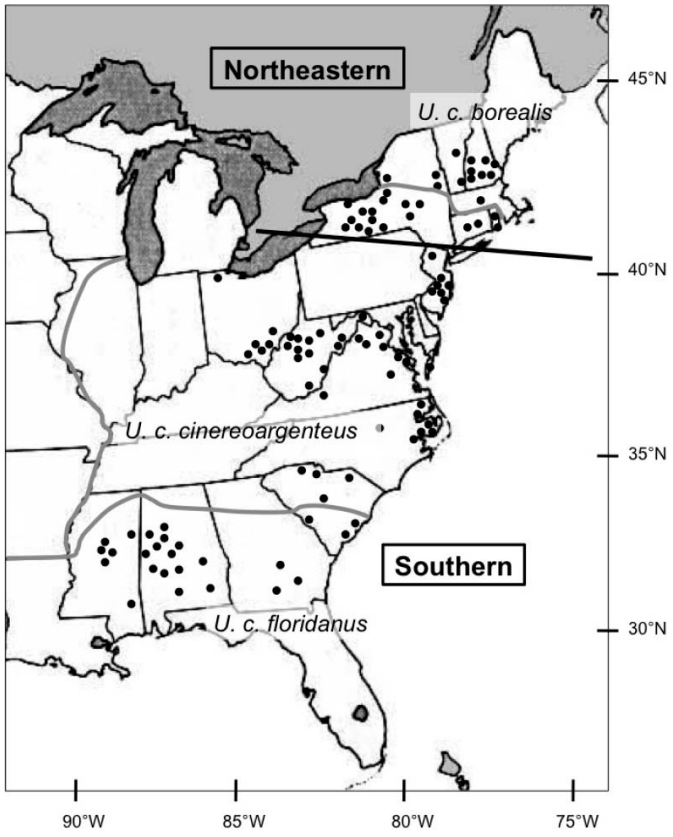

Fig. 1.—Map of the eastern United States showing sampling locations (black dots) for the gray fox (*Urocyon cinereoargenteus*). The division of samples into northeastern and southern regions is shown with a black line, and subspecies boundaries are marked with gray lines. Locations total 293, but not all are distinguishable due to the size and resolution of the map.

Materials and Methods

Sampling and molecular analyses.—We collected 286 tissue samples from various state wildlife, conservation, and trapping organizations in 15 states representing each of the 3 recognized subspecies (Fig. 1). We also included 7 scat samples from Virginia that were part of a larger study and were verified to be from individual gray foxes by molecular methods (Bozarth et al. 2010). We sequenced a sample from a western gray fox (*U. c. scottii*) from Arizona and used it as an outgroup. Upon collection, we stored tissues at room temperature in 75% ethanol and then transferred them to −20°C upon arrival at our lab. We stored scats in plastic bags at −20°C. We extracted total genomic DNA from tissue samples using the QIAGEN DNeasy DNA extraction kit and from scat samples using the QIAGEN QIAamp DNA stool kit (Qiagen Inc., Valencia, California). We amplified 411 bp of the mitochondrial control region with universal primers H16498 and L15910 (Kocher et al. 1989) in a volume of 20 μl with 1.0 unit of AmpliTaq Gold (Applied Biosystems Inc., Foster City, California), 1× polymerase chain reaction buffer, 0.2 mM of each deoxynucleoside triphosphate, 0.5 μM of each primer, 2 mM of $MgCl_2$, and 1 μl of template DNA. We used an 11-min denaturation at 95°C, followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 2 min, and finally a 30-min elongation step at 72°C. We cleaned polymerase chain reaction products using ExoSAP (USB Corporation, Cleveland, Ohio) and sequenced products in both directions using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems Inc.) according to manufacturer's recommendations. We purified reactions via centrifugation through Sephadex G-50 columns (Amersham Biosciences, Piscataway, New Jersey). We ran sequences on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems Inc.) and aligned them by eye in Sequencher 4.6 (Gene Codes Corporation, Inc., Ann Arbor, Michigan).

Genetic structure.—We sampled gray foxes from a broad geographic range, including the ranges of all 3 subspecies that occur in the eastern United States. To test for signatures of genetic structure we used the Tamura and Nei distance method, which differentiates between mutation rates for transitions and transversions and between purines and pyrimidines (Tamura and Nei 1993), to calculate a neighbor-joining tree (Saitou and Nei 1987) in PAUP (Swofford 2002) using the genetic distances among haplotypes. We estimated bootstrap values using a neighbor-joining search in PAUP. For neighbor-joining trees we used our single gray fox from Arizona as an outgroup. We created a minimum spanning haplotype network using statistical parsimony in TCS 1.21 (Clement et al. 2000) to visualize potential regional genetic structure. TCS calculates the probability that pairs of haplotypes are similar for all combinations of haplotypes and then joins the most similar haplotypes together into a network where their combined probability is >95% (Templeton et al. 1992).

To test for differences in molecular diversity among subspecies and among regions we performed an analysis of molecular variance (AMOVA), as implemented in Arlequin 1.1 (Schneider et al. 1997), which uses the sequence data of each haplotype and the frequencies of those haplotypes to test for genetic differentiation in designated regions and groups within those regions. Arlequin creates a squared Euclidean distance matrix and partitions the sum of squared deviations from the matrix into hierarchical variance components, which are tested for significance using permutation tests. The AMOVA partitions the genetic variation among and within user-specified groupings. These variance components are used to calculate Φ-statistics, which are analogous to Wright's *F*-statistics. We 1st divided samples into 3 groups by subspecies designations to test for patterns of differentiation. We then divided samples into northeastern and southern regions with samples from the New England states and New York (Connecticut, Massachusetts, New Hampshire, New York, Rhode Island, and Vermont) grouped in the northeastern region and samples from all other states (Alabama, Georgia, Mississippi, North Carolina, New Jersey, Ohio, South Carolina, Virginia, and West Virginia) grouped in the southern region. This division is justified by the limits of the Pleistocene and post-Pleistocene range of gray foxes suggested by the fossil record (Huntington 1959; Kurtén and Anderson 1980; Waters 1965).

We used DnaSP (Liberado and Rozas 2009) to test for pairwise differences among each of the 3 subspecies and then among the northeastern and southern regions using a distance method (F_{ST}). We followed Wright's guidelines to interpret our F_{ST} values, where a value of 0–0.05 indicates little differentiation, 0.05–0.15 moderate differentiation, 0.15–0.25 great differentiation, and >0.25 very great differentiation (Wright 1978).

Molecular diversity.—To compare molecular diversity of gray foxes across the eastern United States we used DnaSP (Liberado and Rozas 2009) to estimate haplotype diversity and nucleotide diversity for 4 levels of organization, the entire eastern United States, the 2 regions (northeastern and southern), the 3 subspecies, and the 13 degrees of latitude sampled. We performed a linear regression of both haplotype diversity and nucleotide diversity for samples divided by latitude to test for a latitudinal trend of molecular diversity. If eastern gray foxes have experienced rapid demographic expansion northward from a refugium in the South, then genetic diversity is expected to decrease as sampling moves away from the source (Rowe et al. 2004). We used the Stirling probability distribution and Bayes' theorem to test for the completeness of our sampling and to uncover any potential sampling size biases (Dixon 2006).

Origins of expansion.—We generated a minimum spanning haplotype network using statistical parsimony in TCS 1.21 (Clement et al. 2000) to identify the origin of demographic expansion. In expanding populations ancestral haplotypes are expected to be at the origin of the expansion, with derived haplotypes more widespread (Templeton 1998). We inferred the relationships of ancestral and derived haplotypes by their position in the haplotype network, where interior haplotypes

are more likely to be ancestral and haplotypes at the tips are likely to be derived (Templeton et al. 1992). Interior haplotypes will have multiple connections to the rest of the network, whereas more derived haplotypes will have only 1 connection to the network. Any alternative connections among haplotypes in the network that are equally parsimonious could be due to homoplasy caused by the high mutation rate of the control region and by the rapid expansion of the gray fox.

Demographic expansion.—To test for evidence of demographic expansion from a southern refugium we calculated Fu's F_S (Fu 1997) in Arlequin 1.1 (Schneider et al. 1997). The F_S statistic uses the observed mean number of nucleotide differences among samples (θ_π) to test whether a significant excess number of recent mutations or rare alleles exists compared to a random neutral sample. A significantly negative F_S value indicates recent demographic expansion. The significance of the F_S statistic is tested by generating random samples under the hypothesis of selective neutrality and population equilibrium based on a coalescent simulation algorithm adapted from Hudson (1990). The *P*-value is obtained from the proportion of random F_S statistics less than or equal to the observed F_S statistic. We used Arlequin to estimate the F_S statistic for the northeastern and southern regions in 1 analysis, and then for the entire eastern United States to investigate signatures of demographic expansion.

We also evaluated the hypothesis of recent (<10,000 years ago) demographic expansion by calculating mismatch distributions of pairwise differences among all haplotypes within each region using a Tamura and Nei distance method, as implemented in Arlequin 1.1 (Tamura and Nei 1993). Arlequin uses the sequence data to estimate the parameter τ (the coalescent time of expansion), which can be used in the formula $\tau = 2\mu t$ where μ is the mutation rate of the control region per base pair per generation and t is the estimated time since demographic expansion (Schneider and Excoffier 1999). We used a generation time of 1 year and a molecular clock estimate of 17.75% mutations per 10^6 generations as previously applied to control region sequences in red foxes by Aubry et al. (2009). We expect a mutation every 13,707 years in a 411-bp sequence and $\mu = 7.30 \times 10^{-5}$ mutations bp^{-1} $year^{-1}$. We estimated τ and then t for each of the 2 regions and then in a separate analysis for the entire eastern United States.

We used DnaSP (Liberado and Rozas 2009) to compare the distribution of pairwise differences in the northeastern and southern regions and then in the entire eastern United States to the theoretical expectations of both changing and stationary demography. If eastern gray foxes have experienced expansion, the distribution of the observed pairwise nucleotide site differences will produce a right-skewed unimodal peak (Rogers and Harpending 1992).

Results

We obtained control region sequences (411 bp) for a total of 229 out of 293 gray fox samples and partial sequences (<411 bp) from an additional 14 samples (see Appendix I for a complete list of sample locations and haplotypes). Five of the 14 partially sequenced samples included enough variability to consider them unique haplotypes, but these samples were not included in the statistical analyses, except in the construction of the neighbor-joining tree (with and without bootstrap values) and the minimum-spanning network. We failed to amplify 41 tissue samples and 5 scat samples. The complete sequence (411 bp) contained 28 transitions and 2 transversions, resulting in 32 haplotypes, none of which have been reported previously. Eastern gray fox haplotypes were distinguished from each other by 1–11 variable sites, whereas eastern gray foxes were differentiated from our western gray fox outgroup by 14–24 variable sites. We submitted generated sequences to GenBank (haplotype Uci1, accession number GU903018; Uci2, GU903028; Uci3, GU903039; Uci4–Uci9, GU903044–GU903049; Uci10–Uci17, GU903019–GU903026; Uci19, GU903027; Uci20–Uci29, GU903029–GU903038; Uci30–Uci33, GU903040–GU903043; Uci34–Uci38, GU903050–GU903054).

Genetic structure.—The neighbor-joining tree did not indicate clear genetic structure, and the neighbor-joining tree with bootstrap values revealed a polytomy and nodes with little support. Because branch support was so low, we could not make any inferences on genetic structure as it relates to sampling location. In addition, we found low support for the western gray fox outgroup despite twice as much differentiation between this haplotype and the eastern gray fox haplotypes, further supporting the hypothesis of little genetic structure in gray foxes. Neither maximum-likelihood nor maximum-parsimony methods of tree reconstruction can be resolved because too few informative sites exist in the sequences (Clement et al. 2000). The TCS haplotype network revealed little genetic structuring across the region and produced a phylogeny with multiple alternative connections (Fig. 2).

Despite the lack of genetic structure indicated in the neighbor-joining trees and minimum-spanning network, a clear geographic division in haplotype frequency and composition was found between the northeastern and southern regions (Fig. 3). Additionally, differentiation might exist among subspecies, causing structure. We tested for genetic differentiation among each of the 3 subspecies and between the 2 regions using AMOVA and found that more genetic variance is distributed among subspecies and among regions than among sampling locations within each subspecies or region (Table 1). Given the higher variance among regions as compared to within regions, coupled with a significant and high Φ_{CT} value as compared to the lower Φ_{SC} value, the northeastern region can be considered differentiated from the southern region. Because the variation among regions is higher than the variation among subspecies, this division into 2 regions is better supported than current subspecies designations. The pairwise F_{ST} for the 3 subspecies is 0.25 ($P < 0.001$) and for the 2 regions is 0.21 ($P < 0.001$), both indicating great differentiation (Wright 1978).

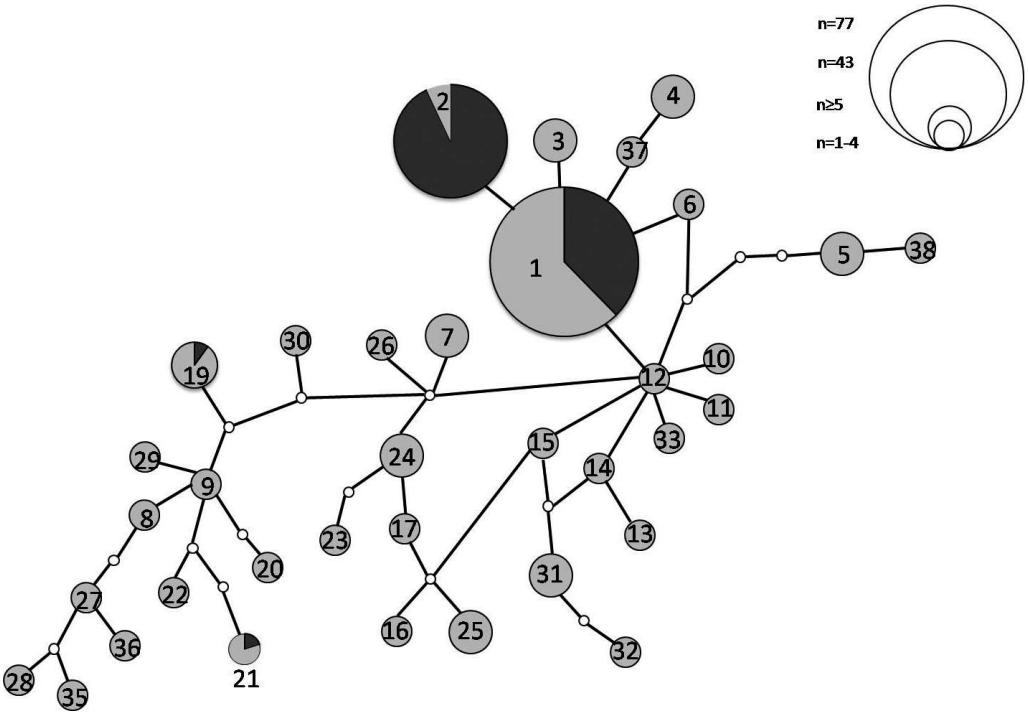

Fig. 2.—Control region minimum-spanning haplotype network for the gray fox (*Urocyon cinereoargenteus*) based on 411 base pairs (bp) for 229 gray fox specimens and partial sequences for an additional 4 specimens. Haplotypes Uci35–Uci38 are partial sequences (<411 bp). Inclusion of haplotype Uci34 produced many alternative connections and was excluded for clarity. Gaps are treated as missing data, not a 5th state. Circle sizes are proportional to the number of individuals represented. Each node represents a 1-bp change. Alternative connections are shown because they could not be resolved with a neighbor-joining tree. The northeastern and southern regions are represented proportionally by dark gray and light gray shading, respectively.

Molecular diversity.—We found a significant trend of decreasing haplotype and nucleotide diversity from the south to the north for both indices (Fig. 4). Our test of adequate sampling (Dixon 2006) for each degree of latitude revealed incomplete haplotype sampling for several degrees of latitude in the southern region. However, we found complete sampling for the northern latitudes, indicating that any further sampling in the South would reveal only more haplotypes and strengthen the relationship between haplotype or nucleotide diversity, or both, and latitude. This trend also is evident when comparing the 3 subspecies; *U. c. floridanus*, the southernmost subspecies, has the highest haplotype and nucleotide diversity, and *U. c. borealis*, the northernmost subspecies, has the lowest values for these indices. The diversity in the entire eastern United States most closely resembles that in the southern region, which contains all of the haplotypes found in the eastern United States (Table 2).

Origins of expansion.—The minimum-spanning network (Fig. 2) shows little genetic structuring between the 2 geographic regions, similar to findings from other studies of North American mammals that do not display regional structure (Helgen et al. 2008; Lance et al. 2003; Lehman and Wayne 1991). Haplotype Uci12 is the most interior haplotype in the network with 8 connections to other haplotypes. Nearly two-thirds (23 of 36) of the haplotypes are completely peripheral, with only 1 connection to the network. Assuming that ancestral haplotypes are internal and derived haplotypes are peripheral (Templeton et al. 1992), Uci12 is ancestral. Haplotypes Uci1 and Uci9 each have 5 connections to the remainder of the network, and notably, Uci1 is clearly the most common haplotype.

Demographic expansion.—We found large, negative, and significant values of Fu's F_S for the southern region and for the entire eastern United States, but a small, positive, and nonsignificant value for the northeastern region (Table 3).

We rearranged the formula $\tau = 2\mu t$ to solve for the time since coalescence for the northeastern and southern regions and the entire eastern United States. Using mismatch distributions of pairwise differences among all haplotypes to estimate τ, and a μ of 7.30×10^{-5} mutations bp^{-1} $year^{-1}$, we estimated a time since coalescence of 4,791 years ago for the northeastern region, but 34,749 years ago for the southern region, which is similar to the 37,696 years ago estimated for the entire eastern United States (Table 3).

The mismatch distribution of pairwise differences within the southern region and also within the entire eastern United States produced a right-skewed unimodal peak that is characteristic of demographic expansion. Because of the extremely low haplotype diversity in the northeastern region, the mismatch distribution analysis was not informative. For both the southern region and the entire eastern United States the observed curve more closely resembles the expected curve, allowing for demographic expansion for which the expected curve does not allow (Fig. 5).

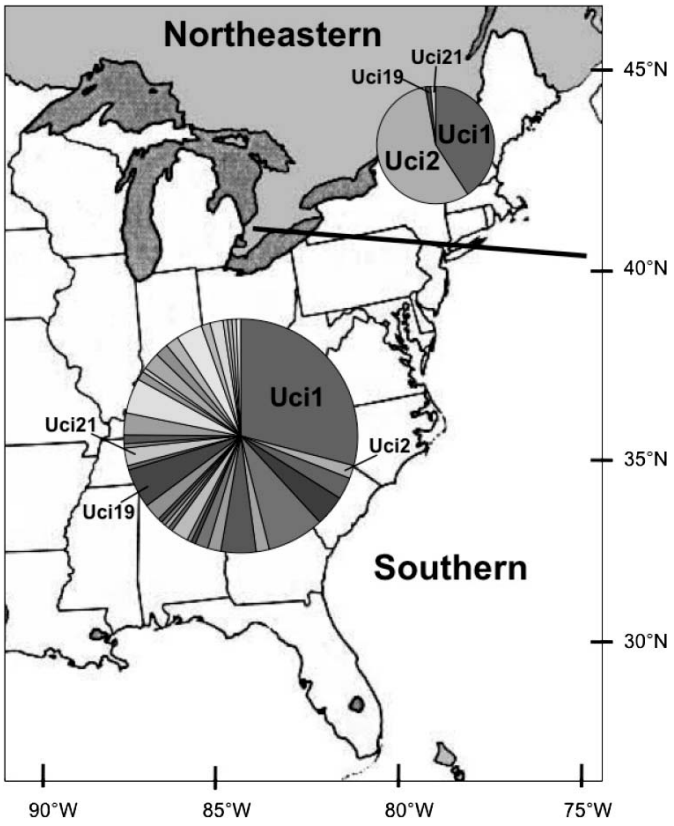

Fig. 3.—Geographic distribution of control region mitochondrial DNA haplotypes among sampled gray foxes (*Urocyon cinereoargenteus*) across the eastern United States. Gray fox sampling locations are divided by the black line into a northeastern and a southern region. Pie charts indicate proportional representation of haplotypes in each region. Some haplotypes are labeled alphanumerically. See Appendix I for more details on haplotype identity and distribution.

Discussion

During the last glacial maximum of the Pleistocene (approximately 18,000 years ago) many North American furbearing mammals were restricted to a refugium in the American Southeast (Arbogast 1999; Aubry et al. 2009; Delcourt and Delcourt 1981; Hayes and Harrison 1992). During this time the Laurentide ice sheet blanketed the northern half of North America, extending south into Pennsylvania between 40°N and 41°N, and into North Carolina along the southern Appalachian ridge to 36°N (Berkland and Raymond 1973). As the climate warmed and the glaciers retreated at the end of the Pleistocene (approximately 10,000 years ago) these mammalian species expanded their ranges northward. For many mammal species anthropogenic translocation and extensive dispersal capabilities can lead to vague signals of genetic structure, with only broad genetic patterns hinting at the history of a species. As evidenced by mtDNA, the red fox (Aubry et al. 2009), black bear (Wooding and Ward 1997), northern short-tailed shrew (Brant and Orti 2003), southern flying squirrel (Arbogast 1999; Petersen and Stewart 2006), northern flying squirrel (Arbogast 1999), eastern chipmunk (Rowe et al. 2004), and eastern woodrat (Hayes and Harrison 1992) have weak genetic structure in eastern North America. Coyotes sampled across the continent also show weak genetic structure (Lehman and Wayne 1991). The red fox, eastern woodrat, and northern flying squirrel in North America have lower nucleotide diversity in sampled regions to the north and east than in more southern and western regions, indicating expansion from Pleistocene refugia. We observed a similar latitudinal pattern in haplotype and nucleotide diversity. We also found evidence of post-Pleistocene expansion from a southern refugium into the Northeast. However, despite very weak genetic structure across the eastern United States, we can use our molecular evidence in conjunction with archaeological and historical records to pinpoint a narrow period of historical hemisphere-wide warming when gray foxes were able to expand into the Northeast.

Gray foxes did not inhabit the northeastern states from the time of the last glacial maximum until as late as 900 (Huntington 1959; Kurtén and Anderson 1980; Waters 1965). Archaeological and historical evidence supports gray fox colonization of the Northeast sometime between 900 and 1635, when the pilgrims observed them in Massachusetts (Guilday 1961a, 1961b; Huntington 1959; Keay 1901). Modern mitochondrial sequences support a division among the northeastern and southern states. Despite weak or no support in the neighbor-joining tree and no clear pattern of

Table 1.—Analysis of molecular variance (AMOVA) results for sampled gray foxes divided into 3 subspecies and into 2 regions (northeastern and southern).

Source of variation	*d.f.*	Sum of squares	Variance of components	Percentage of variation	Fixation indices	*P*
Subspecies						
Among subspecies	2	49.926	0.338	18.31	$\Phi_{CT} = 0.183$	< 0.0001
Among sampling locations within subspecies	117	198.449	0.214	11.61	$\Phi_{SC} = 0.142$	< 0.0001
Within sampling locations	109	141.083	1.294	70.08	$\Phi_{ST} = 0.300$	< 0.0001
Total	228	389.459	1.847			
Regions						
Among regions	1	41.816	0.417	21.35	$\Phi_{CT} = 0.214$	< 0.0001
Among sampling locations within regions	118	206.559	0.241	12.35	$\Phi_{SC} = 0.157$	< 0.0001
Within sampling locations	109	141.083	1.294	66.29	$\Phi_{ST} = 0.337$	< 0.0001
Total	228	389.459	1.952			

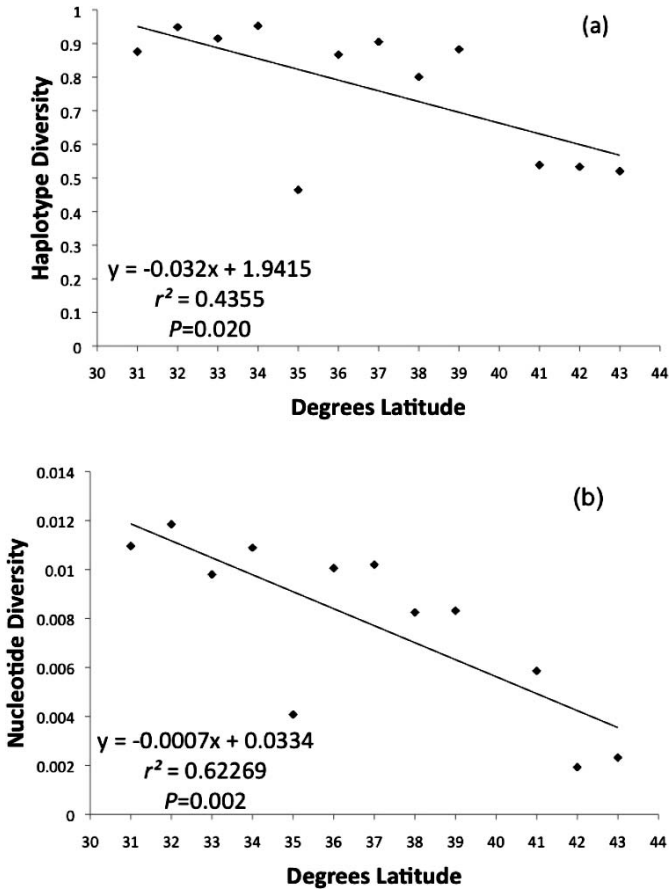

Fig. 4.—Linear regression of a) haplotype diversity and b) nucleotide diversity by latitude for the gray fox (*Urocyon cinereoargenteus*). Samples are divided by degrees latitude from 31°N to 43°N, excluding 40°N, which was represented by only 1 sample.

Table 2.—Indicators of molecular diversity of *Urocyon cinereoargenteus* for 4 levels of organization: the entire eastern United States, the 2 regions (northeastern and southern), the 3 subspecies, and the 12 degrees of latitude sampled (latitude 40°N was not included because it had only 1 sample).

Region	Sample size	No. haplotypes	Haplotype diversity	Nucleotide diversity
Eastern United States	229	32	0.84050	0.00882
Regions				
Northeastern	71	4	0.52728	0.00199
Southern	158	32	0.88658	0.00969
Subspecies				
U. c. borealis	39	3	0.40351	0.00170
U. c. cinereoargenteus	130	23	0.78912	0.00745
U. c. floridanus	60	26	0.93898	0.01083
Latitude				
31°N	18	10	0.87582	0.01096
32°N	13	9	0.94872	0.01185
33°N	30	14	0.91494	0.00980
34°N	7	6	0.95238	0.01089
35°N	8	3	0.46429	0.00408
36°N	6	4	0.86667	0.01006
37°N	7	5	0.90475	0.01020
38°N	31	10	0.80040	0.00825
39°N	36	13	0.88254	0.00832
41°N	13	5	0.53846	0.00586
42°N	34	3	0.53298	0.00193
43°N	26	3	0.52000	0.00232

regional differentiation in a minimum-spanning network, a visual examination of haplotype distribution across the eastern United States reveals unmistakable differences in haplotype composition and frequency between the Northeast and the rest of the sampled area (Fig. 3). The northeastern and southern regions are genetically differentiated as supported by a higher percent variance and higher Φ-statistic among regions than within regions, and by a pairwise F_{ST} value indicating great differentiation. This division into 2 regions is more strongly supported by the AMOVA than is division by subspecies. The northeastern region is characterized by lower haplotype and nucleotide diversity when compared to the southern region. These same values for the southern region reveal more diversity and also are more similar to these indicators of molecular diversity for the entire eastern United States. These findings add support to the archaeological and historical evidence of the relative recent timing of the gray fox colonization into the Northeast.

The most internal haplotype on the minimum-spanning network is haplotype Uci12 with 8 connections. Uci12 was found in 3 foxes in New Jersey and 1 in West Virginia and occurs at a similar frequency to most other detected haplotypes. Haplotypes Uci1 and Uci9 have the 2nd most connections to the network at 5. Notably, Uci1 is the most common haplotype in the entire eastern United States and is very widespread, occurring in 77 of 234 individuals from 11 of the 15 states included in this study. Although more individuals with the Uci12 haplotype might have been detected with increased sampling (especially in Pennsylvania), it is unlikely that the extreme frequency difference between Uci12 and Uci1 would be changed. Because Uci12 occupies the most internal node in the minimum-spanning network, it is most likely the ancestral haplotype (Templeton et al. 1992). However, Uci1 is only 1 mutational step from Uci12 and is undoubtedly the most widespread haplotype. This evidence, combined with the extensively branching topography of the network and the existence of alternative connections, suggests a rapid demographic expansion from Uci12, with Uci1 expanding most prolifically. The dominant presence of Uci1 and Uci2 in the northeastern region could have resulted from a leptokurtic pattern of dispersal, where a few long-distance dispersers on the leading edge of an expansion colonize a new area, leading to modern signatures of reduced genetic diversity (Hewitt 2000). These indicators of reduced molecular diversity are evident in the northeastern region (Table 2). In these expansions the ancestral form, at the rear edge of this movement, is likely to experience a reduction in frequency due to the effects of genetic drift. For gray foxes in the eastern United States this probably has resulted in a reduced range and prevalence of the ancestral haplotype, Uci12.

TABLE 3.—Estimated demographic expansion parameters for each region (northeastern and southern) and for the entire eastern United States. τ is the coalescent time of expansion.

Region	Sample size	No. Haplotypes	Fu's F_S (P)	τ	Estimated expansion time (years ago)
Northeastern	68	4	0.605 (0.604)	0.699	4,791
Southern	165	32	−11.801 (0.002)	5.070	34,749
Eastern United States	233	32	−12.201 (0.005)	5.500	37,696

Evidence of a northward post-Pleistocene demographic expansion of mammals from a southern refugium in eastern North America abounds (Arbogast 1999; Aubry et al. 2009; Hayes and Harrison 1992). For gray foxes in both the southern region and the entire eastern United States Fu's F_S statistic is negative and significant. The mismatch distribution of pairwise differences in the southern region and the entire eastern United States produces the right-skewed, unimodal peak that is characteristic of demographic expansion. According to our estimates using a simple coalescence equation, the time of expansion for the southern region occurred in the late part of the Pleistocene, approximately 34,749 years ago and for the entire eastern United States approximately 37,696 years ago. These dates are likely overestimates of the date of the split because it has been demonstrated that mtDNA polymorphism can be generated before sister populations diverge (Avise 2000). The similarity of date estimates for the southern region and the entire eastern United States further indicates that the expansion originated in the southern region.

It is not surprising to see evidence of a late Pleistocene expansion into the northeastern states from the southeastern United States. However, gray fox colonization of the Northeast appears to be much more recent than 10,000 years ago. Our estimated time of expansion is 4,791 years ago, which is probably an overestimate (Avise 2000). Additionally, this estimate assumes that the present haplotypes have evolved in the region, and the fossil record suggests that they were recent migrants, 1st appearing between 900 and 1600 on Martha's Vineyard, Massachusetts (Huntington 1959). If these haplotypes had originated in the Northeast, it would have taken them approximately 4,791 years to evolve to the observed composition and frequency. However, the more parsimonious explanation is that the haplotypes found in the Northeast evolved in the South and then expanded into the Northeast.

Drawing a parallel between climatic conditions in the 20th century and during a historical period of hemisphere-wide warming, we hypothesize that gray foxes expanded their range into the northeastern region during the Medieval Climate Anomaly, between 800 and 1300, moving with a northern expansion of the deciduous forest, then retracted their range southward during the Little Ice Age, between 1500 and 1850, and finally recolonized the Northeast in modern times. The appearance of gray foxes in the archaeological record of the northeastern states coincides with a trend of warming around the North Atlantic during the Medieval Climate Anomaly

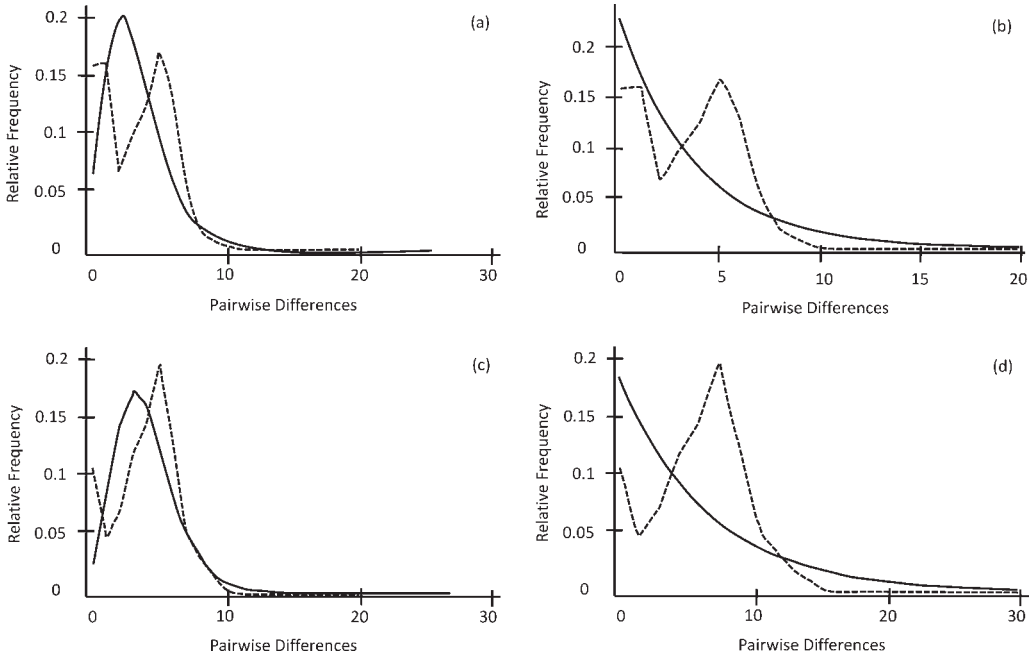

FIG. 5.—Mismatch distribution of pairwise differences of haplotypes for the gray fox (*Urocyon cinereoargenteus*) a, b) in the entire eastern United States and c, d) in the southern region. Shown are observed (hashed lines) and expected (solid lines) frequencies obtained under a model allowing for demographic expansion (b, d) and not allowing for expansion (a, c).

(Bradley et al. 2003; Jungclaus 2009) when the average temperature in North America and Europe was as warm as the period of 1901–1970. The Medieval Climate Anomaly was followed by a period of cooling known as the Little Ice Age. Historical records indicate that the gray fox was either rare or absent in the Northeast during the Little Ice Age (Allen 1876; Churcher 1959; Keay 1901; Trapp and Halberg 1975) and subsequently has recolonized the northeastern states along with the warming trend of the last century (Godin 1977; Palmer 1956). At the end of the Pleistocene the Northeast was dominated by boreal forest, with more temperate mixed hardwood forest to the south (Delcourt and Delcourt 1981). The native red fox inhabited the boreal regions of North America (Kamler and Ballard 2002), whereas the gray fox currently is recognized as very closely associated with deciduous forest (Fritzell and Haroldson 1982). Changes in climate over time cause range shifts for major vegetation types (Woodward 1987), and range shifts for assemblages of floral and faunal communities have been detected during the most recent period of climate change (McCarty 2001). Complex biotic and abiotic interactions make it difficult to conclude that gray foxes and native red foxes have tracked the range changes of deciduous and boreal forests since the Pleistocene. However, knowledge of the habitat preference of each fox species, the shifting of those habitat types, and the fossil record of the 2 species suggests a strong correlation between range shifts for both foxes and forest types.

Although the gray fox remains strongly associated with the deciduous forest (Fritzell and Haroldson 1982), the red fox no longer is limited to the boreal forest (Kamler and Ballard 2002). Unlike the period from the Pleistocene to the arrival of Europeans, the gray fox and red fox are now widely sympatric (Hall 1981), with the gray fox thought to dominate the red fox due to its increased omnivory (Cypher 1993; Hockman and Chapman 1983) and ability to climb trees to escape predation (Fritzell and Haroldson 1982). Red foxes that currently inhabit the eastern United States are either descendants of the European red foxes that were introduced to Virginia in the mid-17th century and to Massachusetts in the mid-18th century (Kamler and Ballard 2002) or potentially native red foxes that have colonized southward from eastern Canada (M. J. Statham, University of California, pers. comm.). The close association of the red fox with boreal forest and the gray fox with deciduous forest is not apparent in recent decades and probably is due to some combination of anthropogenic influences.

Furthermore, we cannot discount the possibility that the range contraction for the gray fox out of New England was due to anthropogenic deforestation in the region. The application of European-style agriculture perpetuated massive deforestation efforts across New England from the settlement of the Pilgrims in the mid-1600s until around 1830, when the process slowed and was eventually reversed (Pfaff 2000). The period of deforestation roughly coincides with the Little Ice Age, and the subsequent reforestation coincides with the modern warming period, complicating differentiation between these 2 potential causes for the change in the range of the gray fox.

Current genetic, fossil, and historical evidence cannot resolve the precise origins of gray foxes in the northeastern states. The molecular data presented in this study cannot pinpoint a narrow enough time frame to distinguish between colonization during the Medieval Climate Anomaly and colonization after the Little Ice Age. Because of their absence from fossil and historical records during the Little Ice Age, modern gray foxes in the Northeast likely are descendants of an expansion that occurred in the last 150 years. However, it is also possible that they are descendants of animals that colonized the Northeast during the Medieval Climate Anomaly and survived the Little Ice Age undetected.

Molecular evidence presented in this study verifies the recent expansion of gray foxes from a refugium in the southern region into the northeastern region. Signatures of post-Pleistocene expansion, including a pattern of high diversity in the south and low diversity in the north, negative and significant Fu's F_S statistics, and unimodal mismatch distributions are similar to the pattern for other mammals in eastern North America. We analyzed genetic, fossil, and historical data, which collectively support the hypothesis that the gray fox did not appear in the Northeast until well after the end of the Pleistocene, during the Medieval Climate Anomaly. As the fossil record becomes more complete we might be able to pinpoint whether modern gray foxes in the Northeast are descendants of those that colonized the region during the Medieval Climate Anomaly or were more recent colonists.

Acknowledgments

Financial and logistical assistance was provided by the Savannah River Ecology Laboratory through contract DE-FC09-07SR22506 from the United States Department of Energy to the University of Georgia Research Foundation and by the Colby College Department of Biology, the Dean of Faculty Student Special Projects Award, and IDeA Networks of Biomedical Research Excellence grant P20 RR-016463. We thank J. G. Germaine for coordination of sample collection and DNA extraction; J. D. Schrecengost, J. C. Kilgo, K. V. Miller, and H. S. Ray for tissue collection; and G. Syed, F. Hailer, C. Hofman, C. W. Edwards, and L. L. Rockwood for assistance and guidance. Thanks are extended to the many state wildlife officials and trappers who helped us obtain specimens and to 2 anonymous reviewers whose comments greatly improved this manuscript.

Literature Cited

Allen, J. A. 1876. The former range of some New England carnivorous mammals. American Naturalist 10:708–715.

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

Arbogast, B. S. 2007. A brief history of the New World flying squirrels: phylogeny, biogeography, and conservation genetics. Journal of Mammalogy 88:840–849.

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.

AVISE, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, Massachusetts.

BERKLAND, J. O., AND L. A. RAYMOND. 1973. Pleistocene glaciation in the Blue Ridge Province, southern Appalachian Mountains, North Carolina, Science 181:651–653.

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.

BRADLEY, R. S., M. K. HUGHES, AND H. F. DIAZ. 2003. Climate in medieval time. Science 302:404–405.

BRANT, S. V., AND G. ORTI. 2003. Phylogeography of the northern short-tailed shrew, *Blarina brevicauda* (Insectivora: Soricidae): past fragmentation and postglacial recolonization. Molecular Ecology 12:1435–1449.

CHURCHER, C. S. 1959. The specific status of the New World red fox. Journal of Mammalogy 40:513–520.

CLEMENT, M., D. POSADA, AND K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

CYPHER, B. L. 1993. Food item use by three sympatric canids in southern Illinois. Transactions of the Illinois State Academy of Science 86:139–144.

DELCOURT, P. A., AND H. R. DELCOURT. 1981. Vegetation maps for eastern North America: 40,000 years B.P. to the present. Pp. 123–165 in Geobotany II (R. C. Romans, ed.). Plenum, New York.

DIXON, C. J. 2006. A means of estimating the completeness of haplotype sampling using the Stirling probability distribution. Molecular Ecology Notes 6:650–652.

ELLSWORTH, D. L., R. L. HONEYCUTT, N. J. SILVY, J. W. BICKHAM, AND W. D. KLIMSTRA. 1994. Historical biogeography and contemporary patterns of mitochondrial DNA variation in white-tailed deer from the southeastern United States. Evolution 48:122–136.

FRITZELL, E. K., AND K. J. HAROLDSON. 1982. *Urocyon cinereoargenteus*. Mammalian Species 189:1–8.

FU, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

FULLER, T. K., AND B. L. CYPHER. 2004. Gray fox *Urocyon cinereoargenteus*. Pp. 92–97 in Canids: foxes, wolves, jackals, and dogs. Status survey and conservation action plan (C. Sillero-Zubiri, M. Hoffman, and D. W. Macdonald, eds.). IUCN Publications, Cambridge, United Kingdom.

GODIN, A. J. 1977. Wild mammals of New England. Johns Hopkins University Press, Baltimore, Maryland.

GUILDAY, J. E. 1961a. Prehistoric record of *Scalopus* from western Pennsylvania. Journal of Mammalogy 42:117–118.

GUILDAY, J. E. 1961b. Vertebrate remains from the Varner site (36-Gr-1). Bulletin of the Society of Pennsylvania Archaeology 31:119–124.

HALL, E. R. 1981. The mammals of North America. 2nd ed. John Wiley & Sons, Inc., New York. Vol. 2.

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.

HELGEN, K. M., J. E. MALDONADO, D. E. WILSON, AND S. D. BUCKNER. 2008. Molecular confirmation of the origin and invasive status of West Indian raccoons. Journal of Mammalogy 89:282–291.

HEWITT, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

HOCKMAN, J. G., AND J. A. CHAPMAN. 1983. Comparative feeding habits of red foxes (*Vulpes vulpes*) and gray foxes (*Urocyon cinereoargenteus*) in Maryland. American Midland Naturalist 110:276–285.

HUDSON, R. R. 1990. Gene genealogies and the coalescent process. Oxford Surveys in Evolutionary Biology 7:1–44.

HUNTINGTON, E. G. 1959. An archaeological study from Martha's Vineyard. Dukes County Intelligencer 1:1–21.

JUNGCLAUS, J. H. 2009. Lessons from the past millenium. Nature Geoscience 2:468–470.

KAMLER, J. F., AND W. B. BALLARD. 2002. A review of native and nonnative red foxes in North America. Wildlife Society Bulletin 30:370–379.

KEAY, F. E. 1901. The animals which our fathers found in New England. New England Magazine 24:535–545.

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.

KURTÉN, B., AND E. ANDERSON. 1980. Pleistocene mammals of North America. Columbia University Press, New York.

LANCE, S. L., J. E. MALDONADO, C. I. BOCETTI, O. H. PATTEE, J. D. BALLOU, AND R. C. FLEISCHER. 2003. Genetic variation in natural and translocated populations of the endangered Delmarva fox squirrel (*Sciurus niger cinereus*). Conservation Genetics 4:707–718.

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.

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

LINZEY, D. W. 1998. The mammals of Virginia. MacDonald & Woodward Publishing Company, Blacksburg, Virginia.

MCCARTY, J. P. 2001. Ecological consequences of recent climate change. Conservation Biology 15:320–331.

MERRIAM, C. H. 1903. Eight new mammals from the United States. Proceedings of the Biological Society of Washington 16:73–77.

PALMER, R. S. 1956. Gray fox in the Northeast. Maine Field Naturalist 12:62–70.

PATTON, J. L., AND P. V. BRYLSKI. 1987. Pocket gophers in alfalfa fields: causes and consequences of habitat related body size variation. American Naturalist 130:493–506.

PETERSEN, S. D., AND D. T. STEWART. 2006. Phylogeography and conservation genetics of southern flying squirrels (*Glaucomys volans*) from Nova Scotia. Journal of Mammalogy 87:153–160.

PFAFF, A. S. 2000. From deforestation to reforestation in New England, United States. Pp. 67–82 in World forests: from deforestation to transition? (M. Palo and H. Vanhanen, eds.). Klumer Academic Publishers, Dordrecht, The Netherlands.

RHOADS, S. N. 1895. New subspecies of the gray fox and Say's chipmunk. Proceedings of the Academy of Natural Sciences of Philadelphia 47:42–44.

ROGERS, A. R., AND H. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

ROWE, K. C., E. J. HESKE, P. W. BROWN, AND K. N. PAIGE. 2004. Surviving the ice: northern refugia and postglacial colonization. Proceedings of the National Academy of Sciences 101:10355–10359.

ROWE, K. C., E. J. HESKE, AND K. N. PAIGE. 2006. Comparative phylogeography of eastern chipmunks and white-footed mice in relation to the individualistic nature of species. Molecular Ecology 15:4003–4020.

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

SCHNEIDER, S., AND L. EXCOFFIER. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.

SCHNEIDER, S., J. M. KUEFFER, D. ROESSLI, AND L. EXCOFFIER. 1997. Arlequin version 1.1: a software for population genetic data analysis. Genetics and Biometry Laboratory, Geneva, Switzerland.

SCHREBER, J. C. D. 1775. Die Säugethiere in Abbildungen nach der Nature mit Beschreibungen. 3rd ed. Walther, Leipzig, Germany.

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

TABERLET, P. 1996. The use of mitochondrial DNA control region sequencing in conservation genetics. Pp. 125–142 in Molecular genetic approaches in conservation (T. B. Smith and R. K. Wayne, eds.). Oxford University Press, Oxford, United Kingdom.

TAMURA, K., AND M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512–526.

TEMPLETON, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Molecular Ecology 7:381–397.

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.

TRAPP, G. R., AND D. L. HALBERG. 1975. Ecology of the grey fox. Pp. 164–148 in The wild canids: their systematics, behavioural ecology and evolution (M. W. Fox, ed.). Van Nostrand Reinhold Co., New York.

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

WATERS, J. H. 1965. Animal remains from some New England sites. Bulletin of the Archaeological Society of Connecticut 33:5–11.

WESTON GLENN, J. L., D. J. CIVITELLO, AND S. L. LANCE. 2009. Multiple paternity and kinship in the gray fox (*Urocyon cinereoargenteus*). Mammalian Biology 74:394–402.

WILSON, D. E., AND D. M. REEDER (EDS.). 2005. Mammal species of the world: a taxonomic and geographic reference. 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.

WISELY, S. M., M. J. STATHAM, AND R. C. FLEISCHER. 2008. Pleistocene refugia and holocene expansion of a grassland-dependent species, the black-footed ferret (*Mustela nigripes*). Journal of Mammalogy 89:87–96.

WOODING, S., AND R. WARD. 1997. Phylogeography and Pleistocene evolution in the North American black bear. Molecular Biology and Evolution 14:1096–1105.

WOODWARD, F. I. 1987. Climate and plant distribution. University of Chicago Press, Chicago, Illinois.

WRIGHT, S. 1978. Evolution and the genetics of populations. University of Chicago Press, Chicago, Illinois. Vol. 4.

Submitted 23 April 2010. Accepted 8 November 2010.

Associate Editor was Burton K. Lim.

APPENDIX I

Sample locations (state, county, town), identification, and haplotypes divided into northeastern and southern regions. Haplotypes represented by a partial (<411 base pairs), but unique, sequence are marked with an asterisk (*). Fecal samples are marked with double asterisks (**). Names of states are represented by postal abbreviations.

Northeastern.—CT, Hartford, Berlin, CT11-CT12, Uci2; CT, Tolland, Hebron, CT41, Uci1; CT, Tolland, Hebron, CT42, Uci2; MA, Worcester, North Brookfield, MA6, Uci2; NH, Belknap, Barnstead, NH1-NH2, Uci21-Uci2; NH, Cheshire, Swanzey Center, NH81, Uci2; NH, Cheshire, West Moreland, NH82-NH83, Uci2; NH, Rock, Chester, NH21-NH22 and NH27-NH28, Uci2; NH, Rock, Rochester, NH23-NH25, Uci2; NH, Rock, Newmarket, NH30-NH31, Uci2; NH, Sullivan, Claremont, NH141, Uci2; NH, Sullivan, Sunapee, NH247-NH250, Uci2; NY, Allegheny, West Almond, NY1, Uci1; NY, Allegheny, Wellsville, NY2 and NY23, Uci1; NY, Allegheny, Andover, NY21 and NY25-NY27, Uci1; NY, Allegheny, Alma, NY22, Uci1; NY, Allegheny, Scio, NY24, Uci1; NY, Allegheny, Angelica, NY27, Uci1; NY, Chemung, Horseheads, NY76 and NY8-NY9, Uci1; NY, Delaware, Colchester, NY97, Uci2; NY, Monroe, Rush, NY71, Uci1; NY, Oneida, Florence, NY28 and NY3, Uci1; NY, Oneida, Camden, NY4 and NY6-NY7, Uci1; NY, Ontario, South Bristol, NY74, Uci1; NY, Oswego, Williamstown, NY5 and NY31-NY32, Uci1; NY, Ostego, Laurens, NY92, Uci1; NY, Ostego, Oneonta, NY93 and NY95-NY96, Uci1; NY, Ostego, Oneonta, NY94, Uci19; NY, Ostego, Oneonta, NY98, Uci2; NY, Saratoga, Moreau, NY101, Uci2; NY, Saratoga, Northumberland, NY102-NY103, Uci2; NY, Steuben, Bath, NY72, Uci1; NY, Steuben, Prattsburg, NY73, Uci1; NY, Steuben, Prattsburg, NY74, Uci2; RI, Bristol, Bristol, RI1, Uci2; RI, Kent, East Greenwich, RI3, Uci2; RI, Kent, Coventry, RI5-RI7, Uci2; RI, Washington, North Kingstown, RI2, Uci2; RI, Washington, Richmond, RI4, Uci2; VT, Orange, Chelsea, VT101, Uci2; VT, Windham, Putney, VT103, Uci2.

Southern.—AL, Bibb, Brent, AL106, Uci7; AL, Bullock, Union Springs, AL102, Uci1; AL, Butler, Greenville, AL48, Uci8; AL, Chilton, Maplesville, AL206, Uci9; AL, Dale, Ozark, AL101, Uci1; AL, Dallas, Cahaba, AL207, Uci22; AL, Hale, Greensboro, AL47, Uci21; AL, Hale, Greensboro, AL49, Uci2; AL, Perry, Uniontown, AL46, Uci7; AL, Pickens, Aliceville, AL204, Uci7; AL, Pickens, Carrolton, AL205, Uci1; AL, Pickens, Reform, AL208, Uci26; AL, Tuscaloosa, Duncanville, AL107, Uci26; AL, Tuscaloosa, Tutwiler, AL88, Uci29; AL, Walker, Goodsprings, AL86, Uci19; AL, Walker, Goodsprings, AL87, Uci17; AL, Walker, Goodsprings, AL89, Uci4; AL, Walker, Goodsprings, AL90 and AL96, Uci21; AL, Walker, Tutwiler, AL100, Uci17; AL, Walker, Tutwiler, AL97 and AL99, Uci7; AL, Walker, Tutwiler, AL98, Uci33; AL, unknown, unknown, AL200, Uci34*; GA, Bacon, Alma, GA66, Uci1; GA, Berrien, Nashville, GA1, Uci31; GA, Berrien, Nashville, GA6 and GA10, Uci8; GA, Berrien, Nashville, GA2-GA3, Uci19; GA, Berrien, Nashville, GA4-GA5, Uci1; GA, Berrien, Nashville, GA7, Uci15; GA, Berrien, Nashville, GA8, Uci23; GA, Pierce, Blackshear, GA68, Uci30; GA, Pierce, Blackshear, GA69, Uci32; GA, Pierce, Blackshear, GA70, Uci1; MS, Attala, Ethel, MS69, Uci16; MS, Attala, Kosciusko, MS67, Uci11; MS, Attala, West, MS58-MS59, Uci1; MS, Greene, Leakesville, MS41, Uci17; MS, Greene, Leakesville, MS42, Uci1; MS, Leake, Carthage, MS66, Uci9; MS, Leake, Carthage, MS68, Uci20; MS, Lowndes, Columbus, MS60, Uci28; NC, Beaufort, Aurora, NC122, Uci29; NC, Beaufort, Bellhaven, NC107, Uci1; NC, Beaufort, Rover, NC123, Uci1; NC, Chowan, Edenton, NC110, Uci30; NC, Chowan, Edenton, NC129, Uci32; NC, Chowan, Edenton, NC130, Uci1; NC, Hyde, Fairfield, NC108, Uci3; NC, Hyde, Middletown, NC124, Uci1; NC, Nash, Rocky Mount, NC109, Uci1; NC, Perquimans, Hertford, NC121, Uci30; NC, Perquimans,

Hertford, NC126, Uci1; NC, Tyrrell, Columbia, NC106, Uci1; NC, Tyrrell, Gum Neck, NC125, Uci1; NJ, Burlington, Browns Mill, NJ10, Uci4; NJ, Burlington, Browns Mill, NJ7, Uci17; NJ, Burlington, Columbus, NJ8, Uci29; NJ, Burlington, Pemberton, NJ3, Uci12; NJ, Burlington, Vincentown, NJ1, Uci25; NJ, Burlington, Vincentown, NJ2, Uci5; NJ, Burlington, Whites Bog, NJ36, Uci2; NJ, Burlington, Woodland Twp., NJ34, Uci1; NJ, Burlington, Woodland Twp., NJ5, Uci12; NJ, Burlington, Woodland Twp., NJ6, Uci21; NJ, Burlington, Woodland Twp., NJ9, Uci33; NJ, Ocean, Barnegat Twp., NJ32, Uci1; NJ, Ocean, Barnegat Twp., NJ33, Uci6; NJ, Ocean, Barnigat Twp., NJ38, Uci1; NJ, Ocean, Barnigat Twp., NJ39, Uci6; NJ, Ocean, Warren Grove, NJ37, Uci6; NJ, Ocean, Whiting, NJ31, Uci31; NJ, Ocean, Whiting, NJ35, Uci12; NJ, Sussex, Stillwater, NJ21, Uci24; OH, Gallia, Vinton, OH13, OH15, and OH56, Uci5; OH, Gallia, Vinton, OH27-OH28, Uci1; OH, Gallia, Vinton, OH29, Uci4; OH, Jackson, Jackson, OH11, Uci5; OH, Lucas, Swanton, OH62, Uci19; OH, Meigs, Langsville, OH57, Uci31; OH, Meigs, Langsville, OH58, Uci5; OH, Scioto, Lucasville, OH59-OH60, Uci5; OH, Washington, Cutler, OH76, Uci4; OH, Washington, Cutler, OH77, Uci5; OH, Washington, Cutler, OH78, Uci2; SC, Aiken, Aiken, GFE7, GFE13, GFRK8, GFRK16-GFRK17, and GFRK19-GFRK20, Uci25; SC, Aiken, Aiken, GFE163, Uci23; SC, Aiken, Aiken, GFE18, Uci35*; SC, Aiken, Aiken, GFE27, Uci36*; SC, Aiken, Aiken, GFE6, Uci28; SC, Aiken, Aiken, GFRK21, Uci7; SC, Charleston, John's Island, SC1-SC2, Uci5; SC, Charleston, Wadmalan Island, SC4, Uci1; SC, Charleston, Wadmalan Island, SC5, Uci3; SC, Cherokee, Gaffney, SC21, Uci9; SC, Marlboro, Blenheim, SC76, Uci28; SC, Marlboro, Blenheim, SC78, Uci14; SC, Richland, Columbia, SC71, Uci1; SC, Richland, West Columbia, SC72, Uci1; SC, York, Rock Hill, SC41, Uci1; SC, York, Rock Hill, SC44, Uci5; SC, York, Rock Hill, SC45, Uci24; VA, Goochland, Centerville, VA27, Uci33; VA, Goochland, Goochland Courthouse, VA26, Uci7; VA, Hanover, Ashland, VA21, Uci37*; VA, King George, Prim, VA28, Uci7; VA, Northumberland, Lewisetta, VA38-VA39, Uci4; VA, Prince William, Manassas, VAgf111704, Uci 1; VA, Prince William, Quantico, VA10.1**, VAB.10**, and VAgf112303, Uci 1; VA, Shenandoah, Edinburg, VA11, VA13, and VA19, Uci1; VA, Shenandoah, Woodstock, VA12, Uci28; VA, Shenandoah, Woodstock, VA18, Uci1; VA, Westmoreland, Horners Beach, VA36, Uci31; VA, Westmoreland, Horners Beach, VA37, Uci4; VA, Westmoreland, Horners Beach, VA40, Uci13; WV, Berkley, Inwood, WV46, Uci12; WV, Braxton, Heaters, WV13, Uci1; WV, Calhoun, Mt. Zion, WV19, Uci19; WV, Gilmer, Ellis, WV11, Uci19; WV, Gilmer, Ellis, WV17-WV18, Uci1; WV, Gilmer, Sand Fork, WV16, Uci3; WV, Greenbrier, White Sulphur Springs, WV49, Uci1; WV, Monroe, Greenville, WV48, Uci19; WV, Monroe, Union, WV47 and WV50, Uci24; WV, Pendleton, Moyers, WV28, Uci31; WV, Pendleton, Sugar Grove, WV26, Uci5; WV, Pendleton, Sugar Grove, WV29, Uci3; WV, Pendleton, Sugar Grove, WV30, Uci38*; WV, Pendleton, Upper Tract, WV36 and WV53, Uci1; WV, Pendleton, Upper Tract, WV37, Uci10; WV, Pendleton, Upper Tract, WV38, Uci3; WV, Pendleton, Upper Tract, WV52, Uci24; WV, Ritchie, Berea, WV31-WV32, Uci19; WV, Ritchie, Berea, WV33, Uci31; WV, Ritchie, Mahone, WV12, WV14-WV15, and WV20, Uci1; WV, Wirt, Elizabeth, WV42 and WV45, Uci1; WV, Wood, Leachtown, WV44, Uci1.