Journal of Mammalogy, 91(5):1112–1123, 2010

Eastern fox squirrel (*Sciurus niger*) lacks phylogeographic structure: recent range expansion and phenotypic differentiation

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The eastern fox squirrel (*Sciurus niger*) occurs naturally over most of eastern North America. The striking patterns of geographic variation in size and coat color displayed by this species are consistent with a hypothesis of southward range contraction and isolation in 2 refugia (in Texas and Florida) during the Last Glacial Maximum, followed by northward range expansion after the glaciers receded. Similar hypotheses have been proposed to explain the patterns in phylogeographic structure exhibited by many plants and animals in eastern North America. We analyzed DNA sequence variation in a 402–base-pair segment of the mitochondrial cytochrome-*b* gene in populations throughout the species' range. Despite our broad geographic sampling, we failed to detect any phylogeographic structure. Unique haplotypes differed from high-frequency haplotypes by only 1 or 2 base pairs, producing a starlike phylogeny of haplotypes. Genetic variation within populations and the species as a whole was characterized by high haplotype diversity and low nucleotide diversity. Taken together, our data indicate that the eastern fox squirrel underwent a rapid range expansion and rapid morphological divergence within the past 14,000 years. DOI: 10.1644/09-MAMM-A-266.1.

Key words: coalescent, cytochrome *b*, glacial refugia, incomplete lineage sorting, mismatch distribution, mitochondrial DNA, phylogeography, Pleistocene, retained ancestral polymorphism, unglaciated eastern North America

Climate change, and the associated changes in size and position of ice sheets, dramatically shifted the distributions of temperate plants and animals in eastern North America during the Pleistocene (Pielou 1991). Phylogeographic data often are used to examine current patterns of genetic connectivity and differentiation to gain insight into historical factors, such as climate change, that have altered the distributions of plants and animals. A growing body of phylogeographic literature (Soltis et al. 2006) now exists for several vertebrate groups, including birds and amphibians, that inhabit unglaciated eastern North America, but Soltis et al. (2006) considered mammals to be underrepresented.

The eastern fox squirrel (*Sciurus niger*) is an ideal candidate for a phylogeographic study of unglaciated eastern North America. This species occurs naturally over most of North America east of the Rocky Mountains, south of about 48°N latitude (Hall 1981; Koprowski 1994). Hall (1981) recognized 10 subspecies, based on marked variation in size and color. Several of these (*S. n. avicennia*, *S. n. cinereus*, *S. n. niger*, and *S. n. shermani*) are listed as threatened or endangered, primarily because of threats to habitat (Loeb and Moncrief 1993).

Size variation in *S. niger* coincides with vegetation type (Weigl et al. 1998). The smallest individuals (average about 700 g) occupy very wet or very dry habitats in disjunct portions of the range (central Texas, the Mississippi River floodplain in Louisiana, and southern Florida), and the largest individuals (average about 1,300 g) occur in southeastern pine forests (Georgia). Animals from predominantly hardwood habitats are intermediate in size.

In addition to striking patterns of size variation, the species displays marked and geographically structured differences in coat color, resulting in 2 distinct but intergrading groups (Weigl et al. 1998; Fig. 1). One group is characterized by reddish, orange, or tan agouti coloration. These animals rarely have white markings on the head, feet, or tail. They occur in south-central Pennsylvania, the Appalachian Mountains, and uplands of the Gulf Coast states, west to the Rocky Mountains and south into central Texas. The other group is typically silver, gray, agouti, or melanic, or a combination of these. They often have tan, gold, or reddish washes over their entire pelage, black markings on their heads, white ears and feet, white or gray noses, and sometimes white-tipped tails. This group is restricted to the Atlantic and Gulf coastal plains

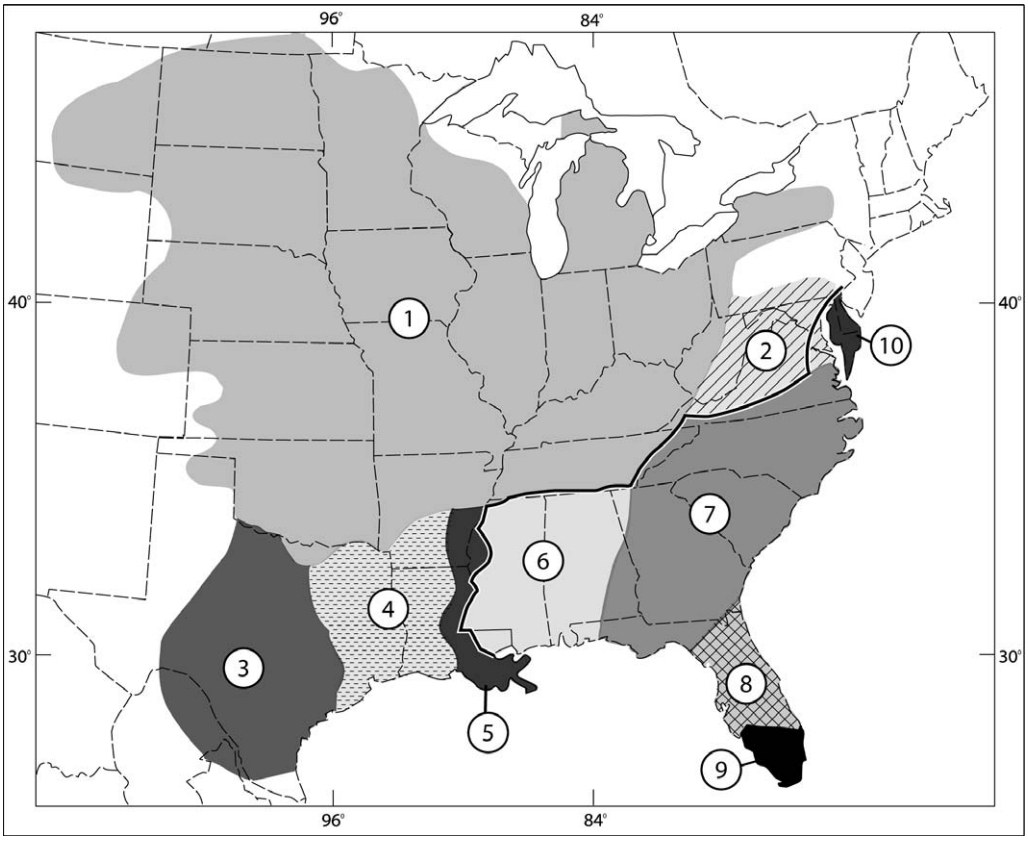

Fig. 1.—Geographic distribution of subspecies of the eastern fox squirrel (*Sciurus niger*—Hall 1981; Koprowski 1994): 1) *S. n. rufiventer*, 2) *S. n. vulpinus*, 3) *S. n. limitis*, 4) *S. n. ludovicianus*, 5) *S. n. subauratus*, 6) *S. n. bachmani*, 7) *S. n. niger*, 8) *S. n. shermani*, 9) *S. n. avicennia*, and 10) *S. n. cinereus*. The heavy dark line distinguishes 2 groupings (1–5 and 6–10) based on coat color. See text for details.

occurring from Delaware south to Florida and west to the lower Mississippi River valley.

Drawing on paleoecological evidence that many temperate species of trees were restricted to southern latitudes during the last ice age and rapidly spread northward following glacial retreat (Davis 1981; Delcourt and Delcourt 1981), Weigl et al. (1998) hypothesized that late Pleistocene glaciations forced populations of *S. niger* into eastern and western refugia. They postulated that the divergent evolution of the 2 major color groups of eastern fox squirrels is the result of an isolation event that lasted for a considerable period of time in forests with different species compositions and climatic conditions. Substantial evidence from a variety of plants and animals (Soltis et al. 2006; Swenson and Howard 2005) supports the existence of 2 refugia, 1 on each side of the Mississippi River.

Moncrief (1993) examined morphologic and allozymic variation in eastern fox squirrels inhabiting the lower Mississippi River valley from central Texas to Mississippi. She demonstrated that morphologic variation does not correspond to allozymic differences but reported allozymic differences among populations of *S. niger* located east and west of the Mississippi River. Subsequent analyses (Moncrief 1998) of additional populations documented the same general east–west patterns of allozymic differences.

The goal of this study was to use DNA sequence data from the mitochondrial cytochrome-*b* gene to investigate relationships among individuals and populations throughout the range of *S. niger* and to assess levels of genetic diversity. We addressed the following questions: does evidence exist for genetic structure in the eastern fox squirrel, and for past or present dispersal and gene flow, fragmentation, isolation, colonization, or range expansion?

Materials and Methods

Sample collection.—We obtained tissue samples from 102 individuals of *S. niger* collected at 18 localities in Arkansas, Florida, Georgia, Indiana, Kansas, Louisiana, Maryland, Mississippi, South Dakota, Texas, and Virginia from 1983 through 2006 (Table 1). All samples were collected following guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). The Florida samples (hairs) were provided by D. Munim, R. Noss, and J. Waterman (University of Central Florida). All other samples (0.5 g of liver) were obtained from the frozen tissue collections of the Virginia Museum of Natural History (VMNH) and Louisiana State University Museum of Natural Sciences (LSUMZ); voucher specimens are deposited in their respective mammal collections. Voucher specimens and tissues of *S. niger cinereus* were transported and are housed at VMNH under Regional Blanket Permit 697823, issued to NDM.

Table 1.—Geographic information and distribution of cytochrome-*b* haplotypes among 18 sampling localities of the eastern fox squirrel (*Sciurus niger*). Locality codes (code) and haplotype designations correspond to those shown in Figs. 2 and 5. For each locality, we provide the number of individuals sequenced (*n*), number of individuals with a haplotype shared by 2 or more localites (A, B, C, D, and P), private haplotypes (with the number of individuals), haplotype diversity (*h*, with standard deviation), nucleotide diversity (π, with standard deviation), Tajima's *D* (with *P*), and Fu's F_s (with *P*).

			Haplotypes									
			Shared									
Code	County(ies) or parish(es) and state	*n*	A	B	C	D	P	Private	*h*	π	Tajima's *D*	Fu's F_s
ARG	Greene, Arkansas	1						O (1)	0.000	0.000		
FLC	Collier, Florida	11				4		U (4) V (3)	0.727 (0.068)	0.003 (0.003)	1.195 (0.904)	1.264 (0.754)
GAB	Baker, Georgia	10		3		5		X (1) Y (1)	0.711 (0.118)	0.004 (0.003)	−0.531 (0.327)	0.017 (0.437)
GAJ	Jasper, Georgia	10		2	1	5		J (1) S (1)	0.756 (0.130)	0.004 (0.003)	−0.229 (0.445)	−1.021 (0.198)
IND	Dubois, Indiana	1			1				0.000	0.000		
KSR	Rooks and Ellis, Kansas	8		1	5		1	H (1)	0.643 (0.184)	0.004 (0.003)	−1.175 (0.152)	−0.519 (0.211)
LAA	Acadia, Louisiana	6			5			K (1)	0.333 (0.215)	0.002 (0.002)	−1.132 (0.149)	0.952 (0.613)
LAB	Bossier, Louisiana	3			3				0.000	0.000		
LAI	East Baton Rouge and Iberville, Louisiana	7	6	1					0.286 (0.196)	0.001 (0.001)	−1.006 (0.235)	0.095 (0.229)
LAM	Madison, Louisiana	5	3					E (2)	0.600 (0.175)	0.003 (0.003)	1.459 (0.944)	1.688 (0.772)
LAS	St. Tammany, Louisiana	3	1	1				G (1)	1.000 (0.272)	0.005 (0.005)		−0.693 (0.146)
MDA	Allegany, Maryland	8		2	5			M (1)	0.607 (0.164)	0.003 (0.003)	−0.525 (0.312)	0.775 (0.634)
MDD	Dorchester, Maryland	8		6	1	1			0.464 (0.200)	0.002 (0.002)	−0.812 (0.254)	0.071 (0.418)
MSH	Holmes, Mississippi	4	1	1			1	T (1)	1.000 (0.177)	0.005 (0.004)	0.273 (0.667)	−1.012 (0.125)
SDC	Clay, South Dakota	2				1		R (1)	1.000 (0.500)	0.007 (0.009)		1.099 (0.449)
TXT	Tom Green, Texas	10	9					N (1)	0.200 (0.154)	0.001 (0.001)	−1.401 (0.083)	0.586 (0.420)
VAA	Alleghany, Virginia	3		1	1			L (1)	1.000 (0.272)	0.005 (0.005)		−0.693 (0.118)
VAS	Sussex, Virginia	2		1				I (1)	1.000 (0.500)	0.002 (0.004)		0.000 (0.246)

DNA extraction, amplification, and sequencing.—Total genomic DNA was isolated from liver samples using standard protocols (Longmire et al. 1997), whereas DNA was isolated from hair samples using the Chelex method (Walsh et al. 1991). The first 402–base-pair sequence of the mitochondrial DNA (mtDNA) cytochrome-*b* gene was amplified using polymerase chain reaction with primers MVZ05 and MVZ04 (Smith and Patton 1991). Double-stranded products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, Wisconsin), and both strands of the purified polymerase chain reaction products were sequenced using Big Dye chain terminators and a 3130 Genetic Analyzer (Applied Biosystems, Inc., Foster City, California). AssemblyLIGN 1.0.9 (Oxford Molecular Group, Oxford, United Kingdom) was used to piece together overlapping fragments for each individual, and CLUSTAL X (Thompson et al. 1997) was used to generate a multiple sequence alignment. MacClade 4.0 (Maddison and Maddison 2000) was used for visual inspection of the multiple sequence alignment and to determine unique haplotypes using the REDUNDANT TAXA option.

Data analysis.—Measures of genetic variation (haplotype diversity and nucleotide diversity) for the total population and within localities were estimated using Arlequin version 3.11 (Excoffier et al. 2007). TCS version 2.1 (Clement et al. 2000) was used to construct a minimum spanning network at a 95% confidence level. Finally, CLUSTAL X was used to align haplotypes of *S. niger* with those of 2 outgroup taxa, as identified by Herron et al. (2004): *Sciurus vulgaris* (GenBank accession number AJ238588) and 2 previously unpublished sequences of *Sciurus carolinensis* (N. D. Moncrief and R. A. Van Den Bussche, pers. obs.). A neighbor-joining tree based on Kimura corrected genetic distances (Kimura 1980) was constructed using PAUP* 4.0b10 (Swofford 2000), and support for internal nodes was estimated using a bootstrap approach with 1,000 iterations.

Arlequin version 3.11 and DnaSP version 4.20.2 (Rozas et al. 2003) were used to generate mismatch distributions, which plot the distribution of pairwise genetic differences between pairs of individuals (Rogers and Harpending 1992). The plots generated from the observed data set were compared to expected distributions under models of sudden expansion (Rogers and Harpending 1992) and constant population size. Also, we calculated Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) using Arlequin version 3.11 to test for selective neutrality, with significant negative values indicating potential recent population expansion. However, mismatch distributions and the aforementioned neutrality tests are incapable of differentiating between recent expansion and selection (i.e., mtDNA selective sweeps—Charlesworth 1992). To differentiate between these processes we also calculated F^* and D^* of Fu and Li (1993) using DnaSP 4.20.2. If F^* and D^* are significant ($P < 0.05$) and F_s is not, selection is indicated. If the reverse is true, the data support recent population expansion (Fu 1997).

In addition to the mismatch distribution we used the coalescent-based approach of the Bayesian skyline plot (Drummond et al. 2005) as implemented in BEAST version 1.4.8 (Drummond and Rambaut 2007). The Bayesian skyline plot estimates effective population size through time, providing a graphical representation of past population demographics. A likelihood ratio test was unable to reject the molecular clock hypothesis for our data set ($2\Delta L = 4.02842$; $P = 0.6728$). Therefore, to produce time estimates in years we used a strict clock and a substitution rate of 7.5–12% divergence per million years under the HKY + I + Γ model of nucleotide substitution. Our analysis consisted of an initial run of 3×10^7 generations, following which operator values were adjusted to optimize search settings. Two final runs of 3×10^7 generations were run with optimized search settings, and the resulting log and tree files were combined to produce final estimates of demographic parameters. All runs were checked for sufficient mixing, stable convergence on a unimodal posterior, and effective sample sizes (Drummond et al. 2002) > 100 for all parameters using TRACER version 1.4 (Drummond and Rambaut 2003).

To date the divergence of *S. niger* and *S. carolinensis* we downloaded from GenBank all available *Sciurus* cytochrome-*b* sequences. The final data set consisted of 1 sequence from each of the following species: *S. niger* (generated for this study), *S. carolinensis* (N. D. Moncrief and R. A. Van Den Bussche, pers. obs.), *S. vulgaris* (AB030028), *S. lis* (AB192923), *S. aestuans* (AJ389530), *S. stramineus* (AB030025), *S. aberti* (SAU10183), and *Glaucomys volans* (AF157921), which was the outgroup. We used a strict clock analysis in BEAST version 1.4.8 (Drummond and Rambaut 2007) with a substitution rate of 7.5–12% per million years (Arbogast et al. 2001). This analysis consisted of an initial run of 2×10^7 generations, following which operator values were adjusted to optimize search settings. Two final runs of 2×10^7 generations were run with optimized search settings, and the resulting log and tree files were combined to produce final divergence estimates.

Results

Among the 102 individuals examined, we detected 22 mtDNA cytochrome-*b* haplotypes (Table 1). Representative sequences of these 22 haplotypes have been deposited in GenBank (accession numbers GU952770–GU952791; Appendix I). Four haplotypes (A, B, C, and D) were widely distributed over the range of the species, occurring at 5, 10, 8, and 5 localities, respectively. Those 4 haplotypes also predominated in the entire data set, occurring in 20, 19, 22, and 16 animals, respectively. A 5th haplotype, P, was present at 2 localities (1 individual at each). Seventeen haplotypes (in a total of 23 individuals) were restricted to a single locality (Table 1; Fig. 2a). Unique haplotypes differed from high-frequency haplotypes by only 1 or 2 base pairs, producing a starlike phylogeny of haplotypes (Fig. 2a). Strong bootstrap support existed for grouping the 22 haplotypes of *S. niger* together; however, relationships among most haplotypes were unresolved (Fig. 2b). Only 2 groupings (haplotypes U and Y,

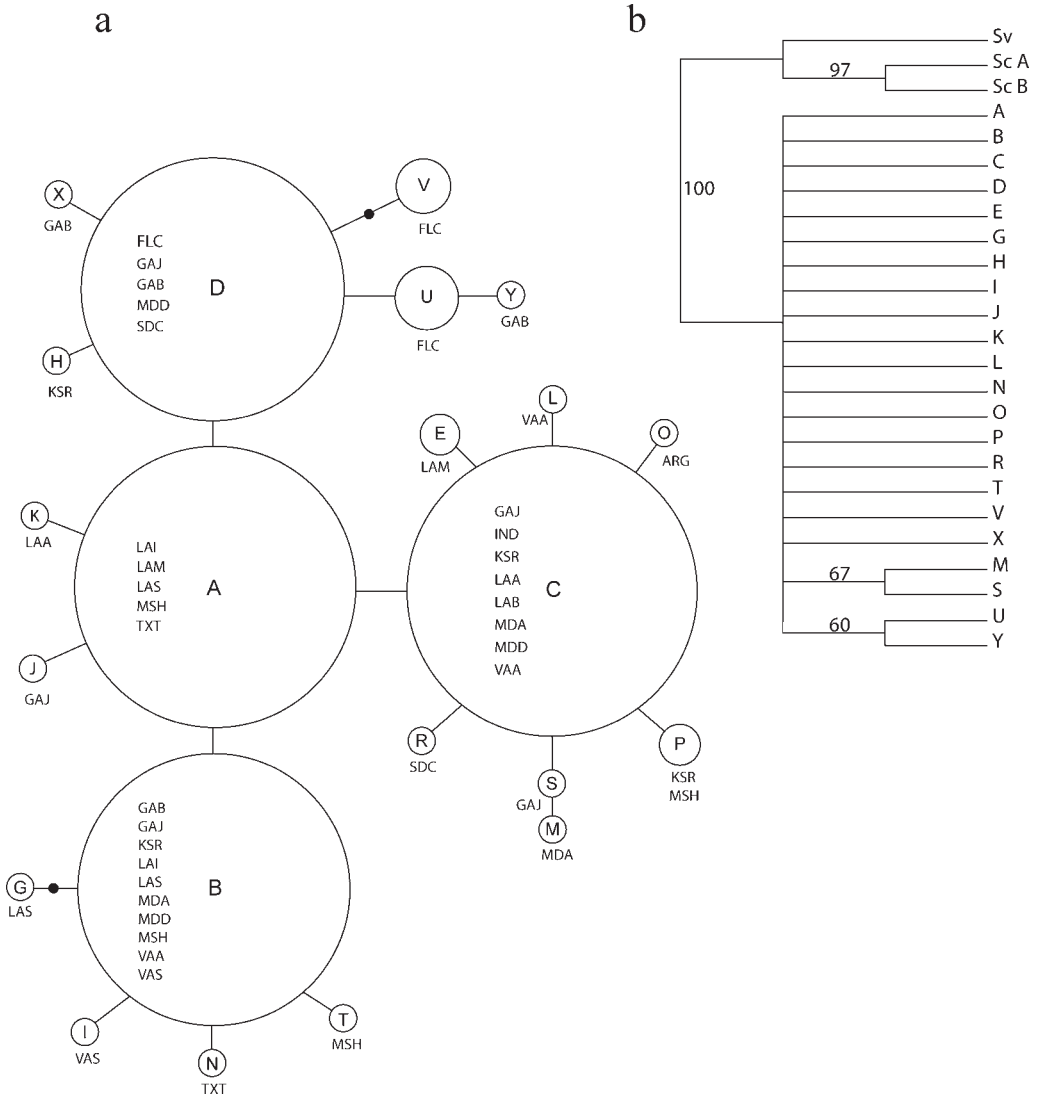

FIG. 2.—a) Parsimony network showing phylogenetic relationships among 22 mitochondrial DNA cytochrome-*b* haplotypes of the eastern fox squirrel (*Sciurus niger*). Haplotype designations (single letters) and codes for sampling localities (3 letters) correspond to those defined in Table 1 and shown in Fig. 5. Each line, regardless of length, represents a single mutational change. The 2 small, filled circles represent unsampled or extinct haplotypes. Size of the labeled circles is proportional to the number of individuals possessing that haplotype. b) Neighbor-joining tree showing phylogenetic relationships among mitochondrial DNA cytochrome-*b* haplotypes of *S. niger*. Two haplotypes (Sc A and Sc B) from *S. carolinensis* (N. D. Moncrief and R. A. Van Den Bussche, pers. obs.) and 1 sequence (Sv, GenBank accession AJ238588) from *S. vulgaris* were used as outgroups. Bootstrap percentages with values > 50 are shown for appropriate nodes.

from localities FLC and GAB, respectively; and haplotypes M and S from localities MDA and GAJ, respectively) have >50 percent bootstrap support. Haplotype diversity for 18 sampling localities of *S. niger* ranged from 0.00 to 1.00, and nucleotide diversity ranged from 0.00 to 0.007 (Table 1).

Mismatch analysis exhibited a unimodal distribution that did not differ significantly from the expected distribution under population expansion (Fig. 3). Harpending's raggedness index was 0.0579 ($P = 0.19$). Fu's F_s was significantly negative (-26.18, $P < 0.01$), and Tajima's D was negative (-1.635, $P = 0.05$), but nonsignificant. Fu and Li's F^* (-3.173, $0.02 < P < 0.05$) was significant, but D^* (-3.732, $0.05 < P < 0.10$) was not. In addition to the mismatch distribution, the Bayesian skyline plot analysis indicated a significant population expansion, which began approximately 14 thousand years ago (kya; Fig. 4). The strict clock dating analysis indicated that *S. niger* and *S. carolinensis* last shared a common ancestor 1.65 million years ago (mya; 95% highest posterior density [HPD] = 2.65–0.85 mya).

DISCUSSION

Despite the broad geographic sampling in this study, we found no phylogeographic structure in the cytochrome-*b* sequences of *S. niger*. We ascribe this lack of structure to incomplete lineage sorting and rapid postglacial range expansion. We detected 4 cytochrome-*b* haplotypes that are shared by populations separated by large geographic distances

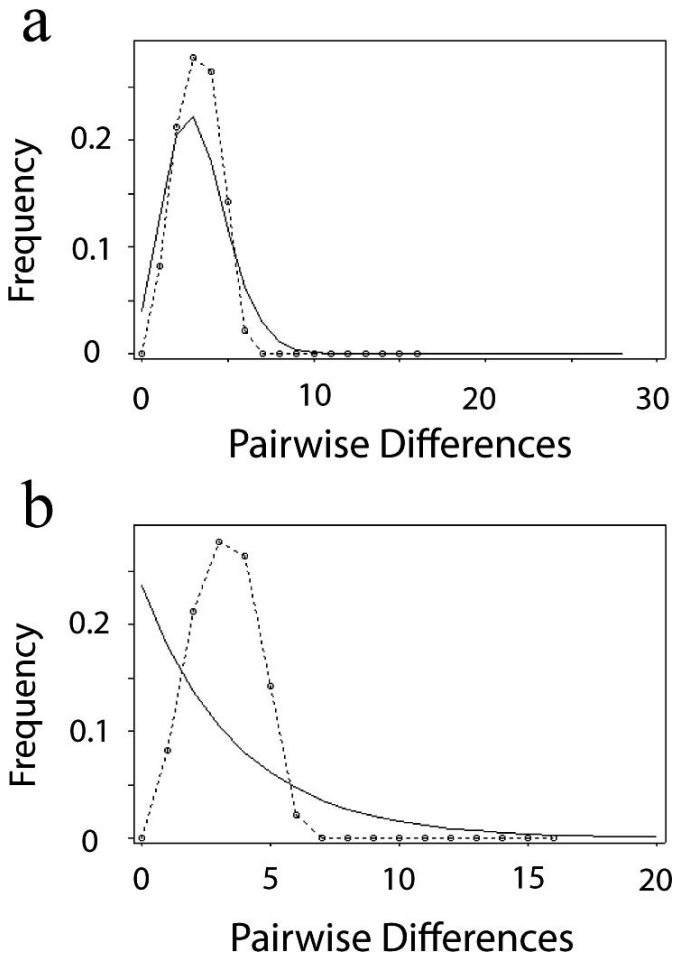

Fig. 3.—Mismatch distributions (dotted lines) for cytochrome-*b* haplotypes of the eastern fox squirrel (*Sciurus niger*): a) expected distribution (solid line) based on a model of exponential population growth; b) expected distribution (solid line) based on a model of constant population size through time.

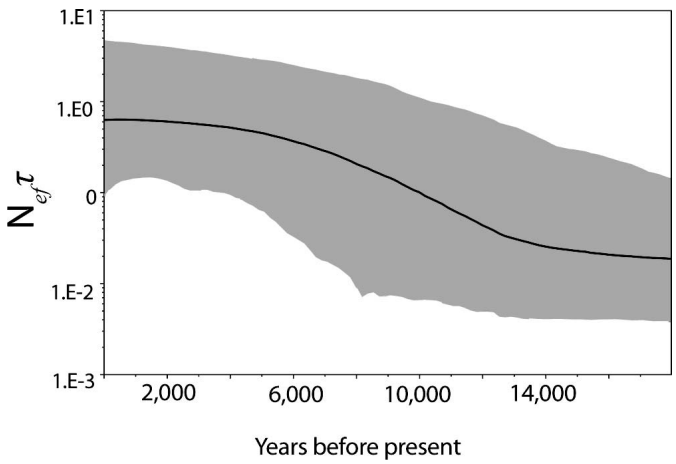

Fig. 4.—Bayesian skyline plot of effective female population size × generation time, $N_{ef}\tau$ (logarithmic scale), for the eastern fox squirrel (*Sciurus niger*). The heavy black line is the median estimate, and the gray shaded area corresponds to the 95% highest posterior density estimate. Time is plotted linearly.

(Fig. 5). The internal position of these high-frequency haplotypes in the parsimony network suggests that they are ancestral maternal lineages that diverged prior to the Pleistocene. We attribute their wide geographic distribution in contemporary populations to rapid growth and expansion of populations of *S. niger* following glacial retreat in the late Pleistocene. Examination of our data suggests that the 2-refugia hypothesis of Weigl et al. (1998), by which a widespread Pleistocene *S. niger* was forced into discrete southwestern and southeastern refugia, is unlikely. Such a scenario would predict an outcome of at least 2 distinct ''stars'' in a haplotype network or strong bootstrap support for at least 2 major groupings of haplotypes, or both. Our data did not sustain either of those outcomes.

The pattern of variation we detected in these data (low levels of geographically unstructured nucleotide diversity) could be due to a selective sweep of adaptive mitochondrial mutations (Charlesworth 1992). However, the nonsignificant D^* and marginally significant F^*, coupled with the highly significant F_s, suggest that the process responsible for this pattern is a recent expansion and not natural selection (Fu 1997). Moreover, Moncrief (1993, 1998) reported relatively low levels of allozymic variation, and Lance et al. (2003) reported low levels of microsatellite polymorphism, providing evidence from unlinked nuclear markers that this species has undergone recent population bottlenecks, or range expansion, or both.

Our data reflect generally high haplotype diversity and low nucleotide diversity within populations and the species as a whole. Another study of mtDNA variation in *S. niger* also reported high haplotype diversity and a lack of phylogeographic structure (Lance et al. 2003). However, the study by Lance et al. (2003) focused on variation within populations on the Delmarva Peninsula, and it lacked the broad geographic sampling of our study.

The combination of high haplotype diversity and low nucleotide diversity is indicative of rapid range expansion after a period of low effective population size (Avise 2000; Grant and Bowen 1998). Rapid population growth enhances the retention of new mutations (Rogers and Harpending 1992), and we documented 17 unique haplotypes, 7 of which are not present at the same locality as the common, ancestral haplotype from which they are derived (Fig. 2a). For example, haplotype H is present only at locality KSR and is only 1 step removed from D; D is not present at locality KSR. Similarly, haplotype R is only present at locality SDC and is 1 step from C; C is not present at locality SDC. Haplotypes K, J, E, O, P, and N are additional examples of this phenomenon.

Our results for *S. niger* contrast sharply with numerous studies that demonstrated substantial phylogeographic structure in many plants and animals from unglaciated eastern North America (Soltis et al. 2006). Most of these studies ascribed phylogeographic structure to 1 or more major biogeographic barriers, including the Mississippi River (Swenson and Howard 2005). Evidence from fossil pollen and plant macrofossils indicates that many temperate species underwent episodes of being pushed into southern refugia

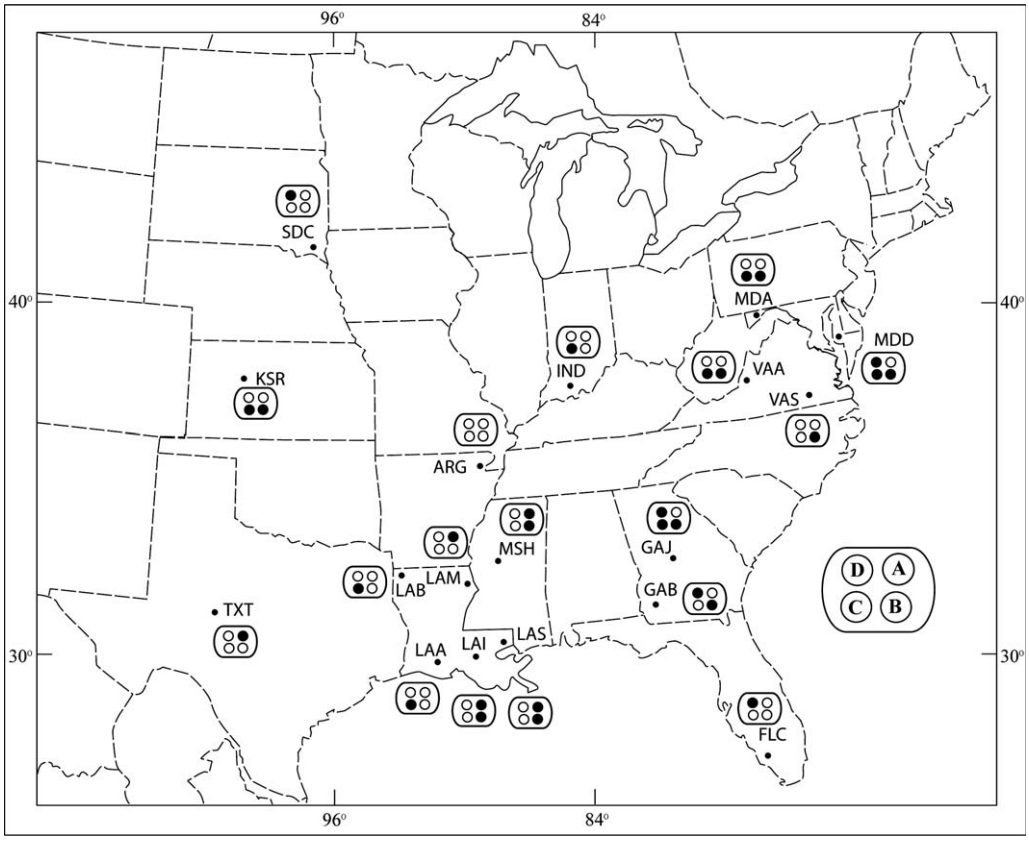

Fig. 5.—Sampling localities and geographic distribution (presence, solid circle; absence, open circle) of the 4 most common cytochrome-*b* mitochondrial DNA haplotypes for the eastern fox squirrel (*Sciurus niger*). Haplotype designations (single letters) and codes for sampling localities (3 letters) correspond to those defined in Table 1 and shown in Fig. 2.

during glacial advances, followed by northward expansion after the glaciers receded (Davis 1981; Delcourt and Delcourt 1981; Soltis et al. 2006). Phylogeographic analyses of annual plants and forest trees indicate that Pleistocene glacial refugia of forest trees existed in central Texas, southern Florida, and the southern Appalachians (Jaramillo-Correa et al. 2009). Alternative scenarios for postglacial spread of these forests suggest that temperate species were present (in ''cryptic'' refugia) in low densities across much of the continent, even during the most severe glacial periods (Bennett 1985; McLachlan et al. 2005).

About 17 percent of the plant and animal species included in the literature survey by Soltis et al. (2006) exhibit no clear phylogeographic structure. At least 2 studies of vertebrates (northern raccoon [*Procyon lotor*—Cullingham et al. 2008] and eastern narrowmouth toad [*Gastrophryne carolinensis*—Makowsky et al. 2009]) that inhabit eastern North America, which were published subsequent to the review by Soltis et al. (2006), report a lack of phylogeographic structure along with high haplotype diversity and low nucleotide diversity.

Our findings that *S. niger* lacks phylogeographic structure are somewhat surprising, considering the striking geographic variation in coat color and size displayed by this species (Weigl et al. 1998). Color variation in eastern fox squirrels can be subject to selection. Kiltie (1992) conducted experiments with models of squirrels from southeastern pine forests and the natural predators of these squirrels (red-tailed hawks [*Buteo jamaicensis*]). He reported that hawks react more slowly to moving squirrel models with patchy black-and-white heads than to squirrel models with plain heads. Kiltie (1992) concluded that squirrels with patchy black-and-white heads might have an adaptive advantage. Thus, geographically related selection for certain colors and color patterns can occur. In contrast, Weigl et al. (1989) concluded that selection is not a factor in maintaining color variation in their study populations in North Carolina because they documented the same 3-to-1 ratio of agouti-to-melanic morphs in adults and litters. Additional research is needed to identify the processes responsible for the patterns of color variation in this species.

In her study of size variation in *S. niger* from the lower Mississippi River valley, Moncrief (1993) outlined potential causes for the small size of eastern fox squirrels that inhabit central Texas and the Mississippi River floodplain in Louisiana. She noted that these size differences can be ecophenotypic (James 1983), or they can represent a completely nongenetic response to trophically poor habitat. Weigl et al. (1989) similarly attributed the small size of animals in southern Florida to poor food resources. Conversely, Weigl et al. (1998) used evidence from extensive studies of feeding behavior and analyses of mandibular characters associated with masseter function to conclude that large body size in some populations of *S. niger* is a selective response for

moving among habitat patches and for handling large food items (e.g., longleaf pine cones) in eastern pine forests. Additionally, Steele and Weigl (1993) presented evidence that large body size in eastern fox squirrels confers a selective advantage for withstanding seasonal periods of food shortage, which are common in the pine forests of the Atlantic and Gulf coastal plains (Weigl et al. 1989).

Whatever the cause (strong regional selection or phenotypic plasticity under different regional environmental conditions), our study of mtDNA variation points to rapid phenotypic differentiation in size and coat color in *S. niger*. A growing body of literature for North American birds (red-winged blackbird [*Agelaius phoeniceus*—Ball et al. 1988], grasshopper sparrow [*Ammodramus savannarum*—Bulgin et al. 2003], and sharp-tailed grouse [*Tympanuchus phasianellus*—Spaulding et al. 2006]) documents rapid range expansions and rapid intraspecific phenotypic diversification since the Last Glacial Maximum. In addition, recent studies have reported a lack of phylogeographic structure in several groups of avian species (juncos in the genus *Junco* [Mila et al. 2007a], warblers in the genus *Dendroica* [Mila et al. 2007b], and prairie grouse in the genus *Tympanuchus* [Johnson 2008]) that exhibit marked phenotypic differentiation within and among closely related taxa.

Examination of our data indicates that eastern fox squirrels underwent a rapid range expansion from a single glacial refugium approximately 14 kya, a date coinciding with a shift towards warmer temperatures and the retreat of the last ice sheet (Bryant and Holloway 1985; Nordt et al. 1994; Ogden 1967; Reid et al. 1970). Examination of our cytochrome-*b* data suggests that *S. niger* diverged from *S. carolinensis* approximately 1.65 mya (95% HPD = 2.65–0.85 mya), a time frame that coincides with the beginning of the Pleistocene glacial cycles (~1.8 mya—Gibbard and van Kolfschoten 2004).

Ancestral haplotypes in *S. niger* were widely distributed geographically, haplotype diversity was not concentrated in any particular area, and haplotype diversity did not decrease with increasing latitutude. These findings preclude identification of the most likely location of the single refugium.

It is noteworthy that a codistributed sciurid, the southern flying squirrel (*G. volans*), displays very little genetic variation over most of eastern North America (Arbogast et al. 2005). Arbogast (2007:845) attributed the relationships among haplotypes within *G. volans* to a ''south-to-north pattern of postglacial recolonization'' out of a southeastern deciduous forest refugium that existed in the Gulf Coast region of North America during the most recent glacial maximum. Another study of phylogeographic structure in *G. volans* (Petersen and Stewart 2006) supported the findings of Arbogast (1999, 2007) and Arbogast et al. (2005) that this species rapidly expanded its range after the Wisconsinan glaciation (about 12 kya). Additional studies of deciduous forest obligates are necessary to test the generality of these results.

Subspecies of *S. niger* are currently delimited based on geographically associated differences in size and coat color. Our study revealed a lack of regional differentiation based on cytochrome-*b* sequence data. These results are in sharp contrast to well-documented regional differences in morphology (Moncrief 1993; Turner and Laerm 1993; Weigl et al. 1998) and ecology (Ditgen et al. 2007; Edwards et al. 1998; Jodice and Humphrey 1992; Kantola and Humphrey 1990; Perkins and Conner 2004; United States Fish and Wildlife Service 1993; Weigl et al. 1989). Additional range-wide studies of *S. niger* that examine segments of DNA that evolve more rapidly and that include nuclear markers (e.g., amplified fragment length polymorphisms) are necessary to reveal patterns of variation beyond the resolution of cytochrome *b* and to examine nuclear patterns of divergence. Those studies, in conjunction with a comprehensive assessment of phenotypic variation, are necessary to assess the taxonomic validity of the currently recognized subspecies.

Implications for conservation.—Populations of *S. niger* in the southeastern United States (*S. n. avicennia*, *S. n. cinereus*, *S. n. niger*, and *S. n. shermani*) are declining, primarily because of habitat destruction and fragmentation (Koprowski 1994; Loeb and Moncrief 1993). Nonetheless, our analyses of cytochrome-*b* sequence data for 18 populations of fox squirrels indicate that genetic variation in *S. n. niger* (GAJ and VAS), *S. n. cinereus* (MDD), *S. n. shermani* (GAB), and *S. n. avicennia* (FLC) is comparable to within-population variation exhibited by fox squirrels at other localities (e.g., LAA and MDA) that presumably have not been affected by recent habitat loss or habitat fragmentation (Table 1).

Examination of our data suggests that habitat loss and habitat fragmentation have not caused a complete loss of genetic variation in *S. n. niger* (GAJ and VAS), *S. n. cinereus* (MDD), *S. n. shermani* (GAB), or *S. n. avicennia* (FLC). These results are consistent with the findings of Moncrief (1998) and Moncrief and Dueser (2001), who used allozymes to examine *S. n. cinereus*. More recently, Lance et al. (2003) documented high levels of haplotype diversity at an mtDNA control region in several populations of *S. n. cinereus* and *S. n. niger*. Although the existence of genetic variation in these southeastern populations of *S. niger* is reassuring, the long-term future of many populations of the eastern fox squirrel remains uncertain. Extensive areas of suitable habitat have been, and continue to be, altered, destroyed, or isolated by land development (Humphrey and Jodice 1992; Loeb and Moncrief 1993; United States Fish and Wildlife Service 1993; Walker et al. 1997).

Acknowledgments

We thank E. Fite, S. Donnelson, and D. Onorato for assistance in the laboratory. We thank D. Munim, R. Noss, and J. Waterman for providing samples for this study, and we thank M. Hafner for allowing access to tissue samples from Louisiana and Mississippi that are housed at LSUMZ. This study was supported, in part, by contract 401814G104 from the United States Department of Interior, United States Fish and Wildlife Service to NDM.

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Submitted 13 August 2009. Accepted 30 March 2010.

Associate Editor was John L. Koprowski.

APPENDIX I

Specimens examined.—Locality code, state, county or parish, voucher number, and haplotype designations for the 102 eastern fox squirrels (*Sciurus niger*) included in this study. Samples without vouchers are marked with asterisks. GenBank accession numbers are provided for each of the 22 haplotypes. Vouchers are housed in the Virginia Museum of Natural History (VMNH) and Louisiana State University Museum of Natural Sciences (LSUMZ).

Locality code	State	County or parish	Voucher no.	Haplotype	GenBank accession no.
ARG	Arkansas	Greene	JAH 467*	O	GU952783
FLC	Florida	Collier	DM 03*	D	GU952773
FLC	Florida	Collier	DM 04*	D	GU952773
FLC	Florida	Collier	DM 08*	D	GU952773
FLC	Florida	Collier	DM 14*	D	GU952773
FLC	Florida	Collier	DM 01*	U	GU952788
FLC	Florida	Collier	DM 02*	U	GU952788
FLC	Florida	Collier	DM 10*	U	GU952788
FLC	Florida	Collier	DM 11*	U	GU952788
FLC	Florida	Collier	DM 09*	V	GU952789
FLC	Florida	Collier	DM 12*	V	GU952789
FLC	Florida	Collier	DM 16*	V	GU952789
GAB	Georgia	Baker	VMNH 0341	B	GU952771
GAB	Georgia	Baker	VMNH 0342	B	GU952771
GAB	Georgia	Baker	VMNH 0350	B	GU952771
GAB	Georgia	Baker	VMNH 0339	D	GU952773
GAB	Georgia	Baker	VMNH 0344	D	GU952773
GAB	Georgia	Baker	VMNH 0345	D	GU952773
GAB	Georgia	Baker	VMNH 0354	D	GU952773
GAB	Georgia	Baker	VMNH 0355	D	GU952773
GAB	Georgia	Baker	VMNH 0338	X	GU952790
GAB	Georgia	Baker	VMNH 0346	Y	GU952791
GAJ	Georgia	Jasper	VMNH 1261	B	GU952771
GAJ	Georgia	Jasper	VMNH 1263	B	GU952771
GAJ	Georgia	Jasper	VMNH 1253	C	GU952772
GAJ	Georgia	Jasper	VMNH 1254	D	GU952773
GAJ	Georgia	Jasper	VMNH 1255	D	GU952773
GAJ	Georgia	Jasper	VMNH 1258	D	GU952773
GAJ	Georgia	Jasper	VMNH 1260	D	GU952773
GAJ	Georgia	Jasper	VMNH 1264	D	GU952773
GAJ	Georgia	Jasper	VMNH 1257	J	GU952778
GAJ	Georgia	Jasper	VMNH 1262	S	GU952786
IND	Indiana	Dubois	VMNH 0326	C	GU952772
KSR	Kansas	Ellis	VMNH 0289	C	GU952772
KSR	Kansas	Ellis	VMNH 0290	P	GU952784
KSR	Kansas	Rooks	VMNH 0296	B	GU952771
KSR	Kansas	Rooks	VMNH 0297	C	GU952772
KSR	Kansas	Rooks	VMNH 0300	C	GU952772
KSR	Kansas	Rooks	VMNH 0301	C	GU952772
KSR	Kansas	Rooks	VMNH 0302	C	GU952772
KSR	Kansas	Rooks	VMNH 0298	H	GU952776
LAA	Louisiana	Acadia	LSUMZ M-5914	C	GU952772
LAA	Louisiana	Acadia	LSUMZ M-7487	C	GU952772
LAA	Louisiana	Acadia	LSUMZ M-7488	C	GU952772
LAA	Louisiana	Acadia	LSUMZ M-7491	C	GU952772
LAA	Louisiana	Acadia	LSUMZ M-7492	C	GU952772
LAA	Louisiana	Acadia	LSUMZ M-7489	K	GU952779
LAB	Louisiana	Bossier	LSUMZ M-2093	C	GU952772
LAB	Louisiana	Bossier	LSUMZ M-2313	C	GU952772
LAB	Louisiana	Bossier	LSUMZ M-7494	C	GU952772
LAI	Louisiana	East Baton Rouge	LSUMZ M-7495	A	GU952770
LAI	Louisiana	Iberville	LSUMZ M-2316	A	GU952770
LAI	Louisiana	Iberville	LSUMZ M-3454	A	GU952770
LAI	Louisiana	Iberville	LSUMZ M-3455	A	GU952770
LAI	Louisiana	Iberville	LSUMZ M-3462	A	GU952770
LAI	Louisiana	Iberville	LSUMZ M-7498	A	GU952770
LAI	Louisiana	Iberville	LSUMZ M-3461	B	GU952771
LAM	Louisiana	Madison	LSUMZ M-2362	A	GU952770

APPENDIX I.—Continued.

Locality code	State	County or parish	Voucher no.	Haplotype	GenBank accession no.
LAM	Louisiana	Madison	LSUMZ M-2363	A	GU952770
LAM	Louisiana	Madison	LSUMZ M-2364	A	GU952770
LAM	Louisiana	Madison	LSUMZ M-2366	E	GU952774
LAM	Louisiana	Madison	LSUMZ M-7499	E	GU952774
LAS	Louisiana	St. Tammany	LSUMZ M-2430	A	GU952770
LAS	Louisiana	St. Tammany	LSUMZ M-2429	B	GU952771
LAS	Louisiana	St. Tammany	LSUMZ M-2431	G	GU952775
MDA	Maryland	Allegany	VMNH 0247	B	GU952771
MDA	Maryland	Allegany	VMNH 0253	B	GU952771
MDA	Maryland	Allegany	VMNH 0249	C	GU952772
MDA	Maryland	Allegany	VMNH 0252	C	GU952772
MDA	Maryland	Allegany	VMNH 0254	C	GU952772
MDA	Maryland	Allegany	VMNH 0255	C	GU952772
MDA	Maryland	Allegany	VMNH 0256	C	GU952772
MDA	Maryland	Allegany	VMNH 0248	M	GU952781
MDD	Maryland	Dorchester	VMNH 1115	B	GU952771
MDD	Maryland	Dorchester	VMNH 1116	B	GU952771
MDD	Maryland	Dorchester	VMNH 1119	B	GU952771
MDD	Maryland	Dorchester	VMNH 1121	B	GU952771
MDD	Maryland	Dorchester	VMNH 1122	B	GU952771
MDD	Maryland	Dorchester	VMNH 1124	B	GU952771
MDD	Maryland	Dorchester	VMNH 1118	C	GU952772
MDD	Maryland	Dorchester	VMNH 1117	D	GU952773
MSH	Mississippi	Holmes	LSUMZ M-2327	A	GU952770
MSH	Mississippi	Holmes	LSUMZ M-2330	B	GU952771
MSH	Mississippi	Holmes	LSUMZ M-2322	P	GU952784
MSH	Mississippi	Holmes	LSUMZ M-2325	T	GU952787
SDC	South Dakota	Clay	VMNH 2385	D	GU952773
SDC	South Dakota	Clay	VMNH 2384	R	GU952785
TXT	Texas	Tom Green	VMNH 0266	A	GU952770
TXT	Texas	Tom Green	VMNH 0276	A	GU952770
TXT	Texas	Tom Green	VMNH 0277	A	GU952770
TXT	Texas	Tom Green	VMNH 0278	A	GU952770
TXT	Texas	Tom Green	VMNH 0279	A	GU952770
TXT	Texas	Tom Green	VMNH 0280	A	GU952770
TXT	Texas	Tom Green	VMNH 0281	A	GU952770
TXT	Texas	Tom Green	VMNH 0282	A	GU952770
TXT	Texas	Tom Green	VMNH 0284	A	GU952770
TXT	Texas	Tom Green	VMNH 0283	N	GU952782
VAA	Virginia	Alleghany	VMNH 0450	B	GU952771
VAA	Virginia	Alleghany	VMNH 0454	C	GU952772
VAA	Virginia	Alleghany	VMNH 0449	L	GU952780
VAS	Virginia	Sussex	VMNH 2275	B	GU952771
VAS	Virginia	Sussex	VMNH 2276	I	GU952777