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Phylogeography of striped skunks (*Mephitis mephitis*) in North America: Pleistocene dispersal and contemporary population structure

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Pleistocene climate fluctuations rearranged ecosystems, and influenced the contemporary distribution of modern species. Although specialist species were often restricted to isolated refugia by Pleistocene climate change, generalist species may have been less constrained in their distribution and movements. We used a combination of genetic data and previously published fossil data to investigate the phylogeography and contemporary population structure of a generalist species, the striped skunk (*Mephitis mephitis*). We sequenced a portion of the mitochondrial cytochrome-*b* gene (601 base pairs) and amplified 8 microsatellite loci from 314 striped skunk specimens. Phylogenetic analysis of the cytochrome-*b* gene revealed the presence of 4 distinct phylogroups, and examination of microsatellite data indicated a pattern of secondary contact among these clades. We infer from these data that during the Rancholabrean stage prior to the Illinoian glaciation, striped skunks emerged from a southern refugium in the Texas–Mexico region and colonized the southeastern United States, forming a 2nd, later refugium in the east. This colonization was followed by a 2nd dispersal event from the southern source population to west of the Rocky Mountains during the Illinoian glacial period. During the Sangamonian interglacial stage, 2 distinct subclades formed on either side of the Sierra Nevada. During the Holocene, the subclade that colonized the Great Basin then expanded east across the northern Rockies and recolonized the Great Plains to create an area of secondary contact with the southern phylogroup. Secondary contact occurred to a lesser extent with individuals from the eastern phylogroup east of the Mississippi River. It appears that periodic Pleistocene glacial expansions and retreats caused a series of range expansions and secondary contact events in this native North American species to create a complex pattern of population structure today.

Key words: generalist species, *Mephitis mephitis*, phylogeography, Pleistocene, population genetics, Quaternary biogeography, striped skunk

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The Pleistocene was a time of periodic climate fluctuations that led to the formation and subsequent retraction of large ice sheets over much of the Northern Hemisphere. The cyclical expansion and contraction of these ice sheets throughout the Pleistocene affected the environment and landscape, and influenced the distribution of continental biota. At the end of the Pleistocene (12,000 years ago), temperatures yet again increased, glaciers retreated, and flora and fauna that had been confined to refugia during the Wisconsinan glacial period expanded as ecosystems developed throughout the Holocene and into recent times (Graham et al. 1996; Hewitt 2000). For many species, Pleistocene glacial cycles and subsequent Holocene climatic warming produced patterns of vicariance and dispersal that, in turn, generated modern patterns of genetic diversity and population structuring in many species of mammals (Hewitt 2004).

Phylogeographic studies, which combine fossil, genetic, climatic, and biogeographic data, seek to understand the vicariant and dispersal processes of plant and animal species that drive Quaternary and contemporary distributions. In North America, phylogeographic studies of fauna have often focused on endemic montane or boreal species that typically expanded out of low-elevation, low-latitude, ice-free Pleistocene refugia to their modern distributions (Lessa et al. 2003;

Lunt et al. 1998; Santucci et al. 1998; Taberlet and Bouvet 1994). Because boreal specialist species depend on a narrow range of resources, species such as the black bear (*Ursus americanus*) and the American marten (*Martes americana*) relied on refugia that were located in the southeastern and eastern United States (Rand 1954; Soltis et al. 2006), the southwestern United States and Mexico (Olah-Hemmings et al. 2010; Rand 1954), and along the Pacific coast (Scudder and Gessler 1989). For some species, eastern Beringia (central Alaska and western Yukon) also functioned as a Pleistocene refugium (Hopkins 1967; Lessa et al. 2003). These refugia served as origins of future range expansion for endemic and specialist species into uninhabited, developing ecosystems following the final retreat of the Laurentide ice sheet at the end of the Wisconsinan glaciation. Generalist species, which were not subject to the same habitat restrictions as specialist species during the Pleistocene, also were relegated to lower latitudes, but they could traverse a broader spectrum of habitats in the unglaciated areas. The greater freedom of movement afforded to generalist species during the Pleistocene may have allowed them to utilize different colonization routes and regions than specialist species (Dragoo et al. 2006; Lister 2004). We investigated the effects of Pleistocene climate change on the distribution and movements of one such North American generalist species, the striped skunk (*Mephitis mephitis*).

Striped skunks are small-bodied mesocarnivores whose modern distribution ranges from northern Mexico through the continental United States and well into Canada (Dragoo 2009; George 2006). All extant skunk species (Mephitidae) in the New World evolved from a primitive skunk genus (*Martinogale*) that migrated to the New World in the late Miocene (Wang et al. 2005); however, 2 basal skunk species in the family, *Mydaus javanensis* and *Mydaus marchei*, are associated with Java and the Philippines, respectively (Dragoo and Honeycutt 1997; Eizirik et al. 2010). Phylogenetic evidence suggests that striped skunks diverged from a common ancestor with their sister genus, *Spilogale* (Dragoo et al. 1993). The earliest fossil evidence of the genus *Mephitis* is from the early Pleistocene (~1.8 million years ago [mya]) and comes from the Broadwater site in Nebraska (Kúrten and Anderson 1980). Late Pleistocene (70,000 years ago to 14,500 years ago) fossil records suggest that striped skunks were broadly distributed across much of the southern half of the United States by the end of the Pleistocene, and fossil records from the Holocene (~10,000-4,500 years ago) provide evidence that striped skunks expanded into the upper Midwest and Northeast regions of the United States following the retreat of the Wisconsinan glacier (FAUNMAP Working Group 1994).

In the current study, we used a combination of genetic, fossil, and biogeographic data to develop a biogeographic history of striped skunks in North America. We hypothesized that, unlike boreal species, the life-history traits of this generalist species afforded striped skunks the ability to move longitudinally during glacial stages when high latitudes and high elevations were uninhabitable, but southern latitudes provided habitat that could be inhabited and provide a route for dispersal. These patterns of movement have been seen in the phylogeographic assessments of such habitat generalists as raccoons (*Procyon lotor*—Cullingham et al. 2008), deer mice (*Peromyscus maniculatus*—Dragoo et al. 2006), and five-lined skinks (*Eumeces fasciatus*—Howes et al. 2006). We hypothesized that following regional dispersal, geographic features such as mountains and major river drainages acted as isolating mechanisms that caused striped skunk populations to diverge into separate lineages. Studies of another highly mobile generalist species, the raccoon, have shown that rivers and mountains are barriers to movement, generating population structure (Biek et al. 2007; Jenkins et al. 1988); in particular, the Mississippi River partitions populations of raccoons (Cullingham et al. 2008), the northern short-tailed shrew (*Blarina brevicauda*—Brant and Orti 2003), and the five-lined skink (Howes et al. 2006). Therefore, we hypothesized that the Mississippi River was a biogeographic barrier to striped skunks both in the Pleistocene and currently. We further hypothesized that climatic warming during the Holocene would have allowed this species to expand latitudinally across North America, and for populations that diverged during the Pleistocene to admix. Studies of striped skunk ecology have shown that they are true habitat generalists (Larivière et al. 1999; Larivière and Messier 1998; Verts 1967) and are highly mobile with a large dispersal capacity (Bixler and Gittleman 2000). Thus, we hypothesized that the biogeographic history of this species would provide evidence for both vicariance and population admixture during the dynamic climatic events of the last 2 million years.

Materials and Methods

Sample collection.—Because striped skunks are a broadly distributed North American species (Hall 1981), we wanted to ensure that we adequately sampled the range of the species. Ear tissue samples from 314 striped skunks were collected by United States Department of Agriculture–Animal and Plant Health Inspection Service employees and the Texas State Health Department from 20 states (Arizona, California, Georgia, Illinois, Indiana, Louisiana, Maine, Michigan, Montana, Nebraska, Nevada, New Mexico, North Dakota, Ohio, Oregon, Texas, Vermont, Virginia, West Virginia, and Wyoming). Samples were sent to Kansas State University for analysis. The full record for each sample included individual identification, state, county, latitude and longitude, GenBank accession, and sex (Appendix I).

Laboratory procedures.—We extracted DNA from 314 ear tissue samples at Kansas State University using a PrepGem Blood and Tissue extraction kit (Zygem, Inc., Solana Beach, California). We amplified a 601–base pair (bp) segment of the cytochrome-*b* gene using 2 primer sets: 31CB (5′-TGAAACTTCGGTTCCTTG-3′) and 728CB (5′-TTCAGTGGATTGGCTGGAGT-3′), and 48CB (5′-GCTCGGAATTTGCTTGATTC-3′) and 745CB (5′-TAATATGGGGTGGGGTGTTC-3′) at a total volume of 10 μl, which consisted of 2 μl of DNA extract (4.5–52.0 ng/μl), 1× polymerase chain reaction buffer,

1.5 mM of $MgCl_2$, 0.2 mM of deoxynucleoside triphosphates, 1.0 μg/μl of bovine serum albumin, 0.5 μM of each primer, and 0.3 unit Taq. Polymerase chain reaction conditions were 55°C for 2 min, followed by 30 cycles of 94°C for 15 s, 50°C for 15 s, and 72°C for 30 s, followed by 72°C for 1 min, ending with a 4°C hold. Polymerase chain reaction products were purified and sequenced at the Advanced Genetic Technology Center (University of Kentucky, Lexington). Sequences were edited using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were deposited in the GenBank database (accession numbers JN008441–JN008709).

We also amplified striped skunk DNA at 8 microsatellite loci (Dragoo et al. 2009) at a total volume of 10 μl, which consisted of 2 μl of DNA extract (4.5–52.0 ng/μl), 1× polymerase chain reaction buffer, 2.7 mM of $MgCl_2$, 0.3 mM of deoxynucleoside triphosphates, 0.1 μg/μl of bovine serum albumin, 0.8 M of Betaine, 0.2 μM of forward primer, 0.5 μM of reverse and 4 dye-tagged primers (FAM and HEX [Integrated DNA Technologies, Coralville, Iowa] and PET and NED [Applied Biosystems, Carlsbad, California]), and 0.5 unit Taq. Polymerase chain reaction conditions for primer sets 22-70, 42-15, 22-19, and 42-73 were 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 51°C for 45 s (primer sets 22-14 and 22-26: 54°C, primer set 42-25: 53°C), and 72°C for 45 s, followed by 10 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 45 s, followed by 72°C for 10 min. Primer set 22-67 required the use of a touchdown polymerase chain reaction, with conditions as follows: 94°C for 5 min, followed by 8 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with the annealing temperature decreasing every 2 cycles to 58°C, 56°C, and 52°C, respectively, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s, followed by 72°C for 10 min. Samples that failed to amplify were reextracted using a DNeasy Blood and Tissue extraction kit (Qiagen Inc., Valencia, California) and reamplified. Polymerase chain reaction product was visualized on an ABI 3730 DNA analyzer (Applied Biosystems) and scored at all 8 loci using GeneMarker version 1.85 (SoftGenetics LLC, State College, Pennsylvania). Each locus was independently amplified and visualized an average of 4 times.

Mitochondrial DNA sequence data analyses.—We based our analyses on a 601-bp section of the cytochrome-*b* gene, and used both graphical and statistical methods of data analysis. Traditional graphical approaches, such as nested clade analysis (Templeton 1998), allowed us to assess population divisions (Templeton 2008). We minimized the risk of committing a type I error that is often associated with graphical analyses by extensively sampling the current distribution of this mobile, yet small-bodied carnivore (Bixler and Gittleman 2000). In order to account for stochastic effects on evolutionary history, we also used a model-based statistical approach to obtain maximum-likelihood estimates of divergence times using IMa2 (available from http://genfaculty.rutgers.edu/hey/software; Hey and Nielson 2007).

We determined the relationships among haplotypes using a median-joining network in Network 4.5.1.6 (available from www.fluxus-engineering.com). The resulting phylogroups formed the basis for all subsequent analyses of historical population. Mutation rates for cytochrome *b* in striped skunks have not been previously calculated; therefore, we relied on known transition and transversion fractions for the family Mustelidae, the sister family to Mephitidae (Marmi et al. 2004). We used these fractions and the date for the 1st known striped skunk fossil (1.8 mya—Kúrten and Anderson 1980) to calculate a mutation rate of 6.20×10^{-8} substitutions per site per year. Assuming a generation time of 1 year in striped skunks, we expected a mutation to occur every 26,837 years on average for a 601-bp sequence. We used this mutation rate to estimate divergence times in terms of average number of mutations (rho) separating ancestral and descendent haplotypes in Network 4.5.1.6 (Forster et al. 1996). We conducted nested clade analysis using the median-joining network generated from the cytochrome-*b* data set in Network 4.5.1.6. We defined nested clades based on the rules of Templeton et al. (1987), and conducted the nested clade analysis using GeoDis 2.4 (available from: http://darwin.uvigo.es/software/geodis.html; Posada et al. 2000) and Templeton's (1998) inference key.

We constructed phylogenetic trees using the Bayesian method implemented in BEAST 1.4.8 (available from http://beast.bio.ed.ac.uk/Main_Page; Drummond and Rambaut 2007), and calculated posterior probabilities from 10^7 iterations after discarding the first 10^5 iterations as burn-in. We used a general time-reversible (GTR) model of DNA substitution, proportion of invariable sites, and shape of the gamma distribution (GTR+I+G model, base frequencies of A = 0.2961, C = 0.2519, G = 0.1353, T = 0.3167, rate matrix = [4.2082, 17.7883, 1.8460, 1.0952, 44.5668], I = 0.4611, G = 0.6328) using MrModeltest 2.3 (available from https://wiki.uio.no/usit/suf/vd/hpc/index.php/Modeltest/MrModeltest; Nylander 2004), to analyze the cytochrome-*b* data set in Bayesian analyses. We used the hooded skunk (*Mephitis macroura*; cytochrome *b*, GenBank accession DQ471840) as our outgroup. A maximum clade credibility tree was generated from 9,001 trees after a burn-in of 1,000 trees in TreeAnnotator 1.4.8 (part of the BEAST package), and visualized using FigTree 1.3.1 (available from: http://tree.bio.ed.ac.uk/software/figtree/).

We used Markov chain Monte Carlo–based simulations in the program IMa2 to assess an isolation-with-migration demographic model for striped skunks, and to produce maximum-likelihood estimates and confidence intervals for divergence times among haplogroups from median-joining networks. We used the Hasegawa, Kishino, & Yano (HKY) model of substitution and the cytochrome-*b* data set for this analysis. We began with multiple runs of 1,000 steps (following 100,000 iterations as burn-in) to assess mixing and to fine-tune the parameter space. We then conducted 2 independent runs of 1,000,000 Markov chain Monte Carlo simulation steps. Consistent marginal peak locations with unimodal likelihood curves approaching 0 on either end of the distribution of parameter values indicated reasonable sampling of trees, which were then used in ''LoadTree'' mode to estimate joint distributions, final parameter estimates, and

credibility intervals (Hey and Nielson 2007). Although generation time to most recent common ancestor (T_{MRCA}) is not analogous to divergence time, we compared values to increase our confidence in divergence time. T_{MRCA} represents the time (in generations) that lineages shared a common relative, while divergence time represents when populations genetically diverged from one another. T_{MRCA} will be older than divergence time; however, the high dispersal capacity of striped skunks combined with Pleistocene climate fluctuations likely led to rapid population structuring, so that the time between the 2 estimates might be short. Thus, we felt that it was appropriate to use T_{MRCA} to confirm our divergence time estimates.

We calculated T_{MRCA} using a Bayesian coalescent-based approach implemented in BEAST 1.4.8, and specified a Bayesian skyline plot as the demographic model (Drummond et al. 2005). Bayesian skyline analysis uses a Markov chain Monte Carlo approach (Drummond et al. 2002), allowing for simultaneous estimation of genealogy, nucleotide substitution rate, and demography. Bayesian skyline analysis as implemented in BEAST is a coalescent model and does not impose a specific demographic growth pattern a priori, because demography is one of the fitted components. We ran the analysis for 10^6 iterations, discarding the first 10^5 iterations as burn-in; we reran the analysis until each scale factor was optimized, which occurred after 7 runs. Our final run consisted of 10^7 iterations, with the first 10^6 iterations discarded as burn-in. We visualized the results with Tracer 1.4.1 (available from http://tree.bio.ed.ac.uk/software/tracer/). We also tested for population growth by calculating Fu's F_S (Fu 1997) in DnaSP 5.5 (available from http://www.ub.edu/dnasp/; Rozas et al. 2003) for different phylogroups as indicated by our haplotype networks and phylogenetic trees. Fu's F_S compares the number of polymorphic sites to the total number of nucleotide differences to detect population growth; populations with recent expansion have statistically significant negative values (Fu 1997).

For geographical analyses, we used SAMOVA version 1.0 (available from http://web.unife.it/progetti/genetica/Isabelle/samova.html) to independently evaluate population structure (Dupanloup et al. 2002). We assumed that the number of geographical groupings (K) ranged from 2 to 5 for cytochrome *b*, based on haplogroups from the median-joining networks. Using multiple geographic groupings allowed us to test hypotheses about the relationships among phylogroups to determine patterns of population structure among striped skunks. We evaluated the degree to which population genetic differences could be explained by isolation by distance in Arlequin 3.11 (available from http://cmpg.unibe.ch/software/arlequin3/) using Mantel tests between pairwise Φ_{ST}, a measure of genetic differentiation among phylogroups and geographical distances among those clades.

We used Arlequin 3.11 to estimate nucleotide and haplotype diversity and to generate a matrix of pairwise Φ_{ST} values based on pairwise differences between haplotypes for cytochrome *b* (Nei and Li 1979). We evaluated statistical significance ($P = 0.05$) based on 1,000 permutations, then performed a sequential Bonferroni correction for multiple tests (Rice 1989).

Microsatellite DNA analyses.—We used 8 microsatellite loci to assess the contemporary population structure of striped skunks. First, we determined the number of segregating populations in our host population using a Bayesian clustering algorithm in STRUCTURE 2.3 (available from http://pritch.bsd.uchicago.edu/structure.html; Pritchard et al. 2000) by conducting 1 run for $K = 1$ through $K = 25$, where K is the number of populations. After the initial runs, we narrowed the population size range and conducted 20 runs each for $K = 1$ through $K = 6$. We determined the likely number of populations based on the change in the log probability of data between K values (Evanno et al. 2005). Individuals were assigned to a population according to the individual q-value (the proportion of each individual's genome that can be assigned to a given population) determined in STRUCTURE; these populations formed the basis for all subsequent contemporary population genetic analyses. We tested for Hardy–Weinberg equilibrium using GENEPOP 3.4 (available from http://genepop.curtin.edu.au/; Raymond and Rousset 1995); to ensure that microsatellite loci were randomly associated with one another, we tested for linkage disequilibrium (GENEPOP 3.4). We estimated mean allelic richness for each population using FSTAT 2.9 (available from http://www2.unil.ch/popgen/softwares/fstat.htm; Goudet et al. 2002). We also determined the global F_{ST} value and population pairwise F_{ST} values to determine the amount of gene flow among populations throughout North America. We used MIGRATE 2.3 (available from http://popgen.sc.fsu.edu/Migrate/Migrate-n.html; Beerli and Felsenstein 1999) to estimate the relative effective population size (θ) of striped skunks throughout North America by calculating $\theta = 4N_e\mu$ for skunk populations. We used a Brownian microsatellite model, and ran the program until the values became stationary. Because striped skunks succumb to a variety of diseases, we also tested all populations for the signature of a bottleneck using BOTTLENECK 1.2.02 (available from http://www1.montpellier.inra.fr/URLB/bottleneck/bottleneck.html; Cornuet and Luikart 1996).

We also investigated the fine-scale population structure of striped skunks using a combination of linear regression and population structure analyses for individuals in the west, central, and eastern United States. Using microsatellite markers, we calculated the average q-value for all individuals in the Great Plains, including animals from Texas, Nebraska, Wyoming, Montana, and North Dakota, and regressed the average q-values against latitude to test the hypothesis that contemporary secondary contact was occurring among separate lineages of skunks in northern and southern populations of the Great Plains (Ward and Neel 1976). We conducted linear regression analysis using SAS (SAS Institute Inc., Cary, North Carolina) to test for the presence of clinal change in allele frequency. To test for secondary contact of distinct lineages east of the Mississippi River, we regressed the average q-values for individuals from Georgia, Virginia, West Virginia, Indiana, Illinois, Ohio, and Michigan against latitude.

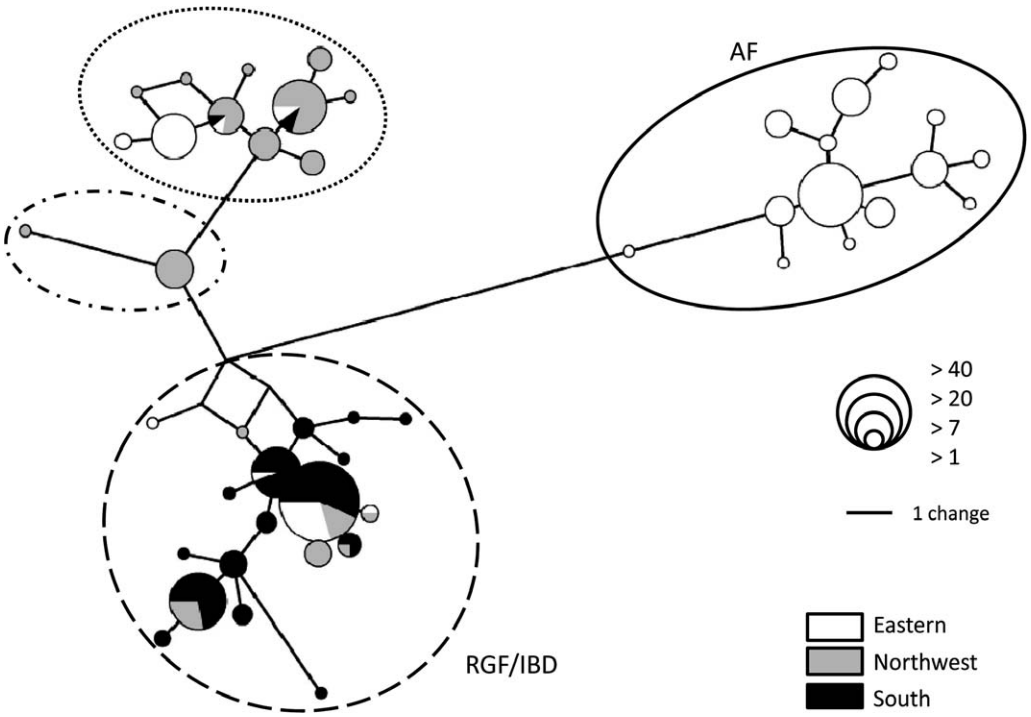

Fig. 1.—Median-joining network based on 601 base pairs of cytochrome *b* in the mitochondrial genome for 269 striped skunk specimens. Branch lengths are proportional to the number of substitutions, and circle sizes are proportional to the number of individuals represented. Ambiguous connections were resolved using the rooted maximum clade credibility phylogenetic tree. The East clade is indicated with a solid line, the Intermountain West clade is indicated with a dotted line, the Pacific clade is indicated with a dot–dash line, and the South clade is indicated with a dashed line. Results of nested clade analysis, including allopatric fragmentation (AF), restricted gene flow (RGF), and isolation by distance (IBD), are indicated where significant.

Results

Nested clade analysis and phylogeny.—We obtained partial cytochrome-*b* sequences (601 bp) from 269 of the 314 samples. The cytochrome-*b* haplotype network revealed that haplotypes were divided into 4 phylogroups. The Pacific phylogroup contained only samples from California; the South phylogroup contained mostly samples from the southwestern and Gulf Coast states (Arizona, New Mexico, Texas, and Louisiana); the Intermountain West phylogroup consisted mostly of samples from the northern Great Plains, Great Basin, northern Rocky Mountains, and Pacific Northwest (Oregon, Montana, Nebraska, Nevada, North Dakota, and Wyoming); and the East phylogroup contained mostly samples east of the Mississippi River (Georgia, Illinois, Indiana, Maine, Michigan, Ohio, Vermont, Virginia, and West Virginia; Fig. 1). Some samples from Montana and Wyoming also were present in the South phylogroup, and some samples from Texas and New Mexico were present among haplotypes found in the Intermountain West group. Additionally, samples from Illinois were also found in both the South and Intermountain West phylogroups.

The nested clade analysis revealed a pattern of contiguous range expansion for the entire network that represented the entire continental sample (Fig. 1). In addition, significant patterns of demography were found for the East and South phylogroups. The East phylogroup showed evidence of allopatric fragmentation; the South phylogroup showed evidence of restricted gene flow and isolation by distance using Templeton's key (1998). Mantel tests using the Φ_{ST} values from the cytochrome-*b* data set and geographic distance confirmed the presence of a pattern of isolation by distance for striped skunks throughout North America ($r^2 = 0.14$, $n = 6$, $P = 0.02$).

The patterning seen in the cytochrome-*b* median-joining network was consistent with the maximum clade credibility phylogenetic tree (Fig. 2). Using hooded skunk as the outgroup, the phylogram showed well-formed clades. The split of the East clade and the South clade was the oldest divergence event, followed by the split of the Intermountain West clade from the South clade. Finally, the Pacific clade split from the Intermountain West clade most recently, and represents the most derived clade. All bifurcation points representing these splits had greater than 0.5 posterior probability support, trees built using maximum parsimony had similar topologies (results not shown), and molecular clock estimates supported the chronology of events.

Results of the isolation-with-migration model, generated by the program IMa2, indicated that the East phylogroup split from the South phylogroup an estimated 209,000 years ago, the Intermountain West phylogroup split from the South phylogroup an estimated 149,000 years ago, and the Pacific phylogroup split from the Intermountain West phylogroup an estimated 132,000 years ago. However, the credibility intervals were broad for all divergence time estimates (Table 1). We corroborated these divergence estimates using 2 other

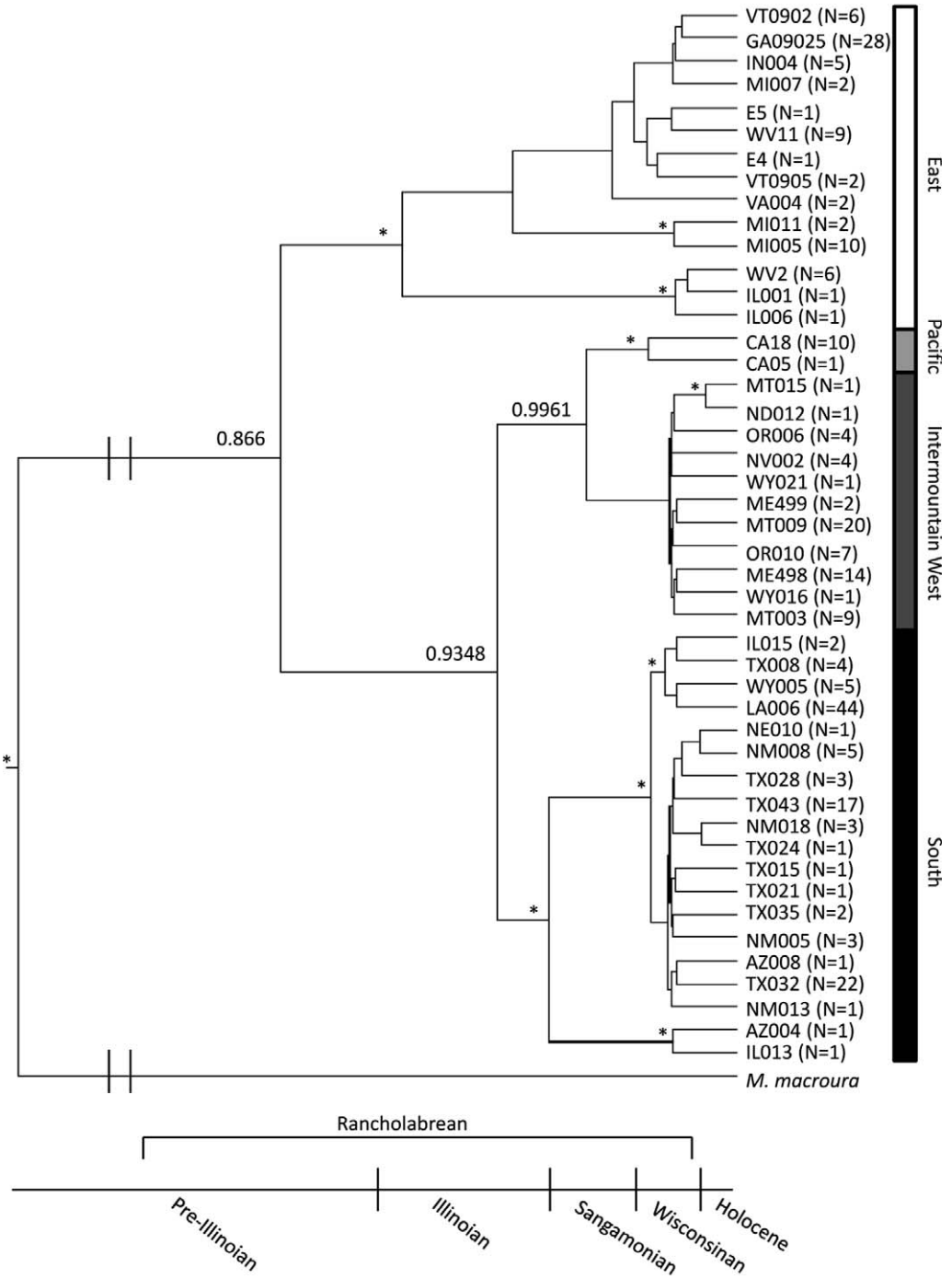

Fig. 2.—Maximum clade credibility chronogram of cytochrome-*b* haplotypes constructed under a GTR+I+G model of evolution based on 601 base pairs for a subset of 46 striped skunk specimens. Asterisks at nodes indicate posterior probability values greater than 0.5; posterior probability values are explicitly indicated at nodes of interest. Numbers in parentheses indicate the number of samples out of 269 that share a unique haplotype. We used hooded skunks (*Mephitis macroura*) as the outgroup. Shaded bars at right indicate phylogroup or clade designation. Branch length for outgroup is not drawn to scale.

methods, the generation T_{MRCA} for each haplogroup in BEAST using a skyline model, and the estimation of rho statistics in Network. The T_{MRCA} values were similar to the splitting time estimates for the isolation-with-migration model generated using IMa2, as were the rho estimates generated in the program Network (Table 1). The demographic model constructed from the entire cytochrome-*b* data set also corroborated our interpretation of the nested clade analysis, indicating steady population size throughout much of the Pleistocene, followed by extensive range expansion by striped skunks in North America following the retreat of the Wisconsin glacier.

Historical population structure of striped skunks in North America.—Results of the SAMOVA indicated signifi-

TABLE 1.—Estimates for phylogroup divergence times using 3 different estimators based on cytochrome-*b* sequences. The 95% credibility intervals for the posterior distributions of isolation with migration and time to most recent common ancestor (T_{MRCA}) are indicated in parentheses.

Splitting event	Isolation with migration (× 1,000 years)	T_{MRCA} (× 1,000 years)	Rho estimate	Rho estimate in years (× 1,000 years)
South–East	209 (123–2,144)	358 (145–602)	10.7 ± 2.52	287 ± 68
South–Intermountain West	149 (87–228)	130 (60–213)	6.11 ± 1.82	164 ± 49
Intermountain West–Pacific	132 (74–207)	97 (17–215)	3.87 ± 1.64	104 ± 44

cant models for both 3 and 4 populations of striped skunks in North America. In general, we found a continental pattern of separation by longitude and latitude. The Φ_{ST} values were similar for all 4 levels of population structure, but the Φ_{ST} value for 4 populations was higher than for 3 populations and was an order of magnitude more significant, suggesting that 4 was most likely the correct number of groupings. Samples west of the Rocky Mountains and in the northern and central Great Plains defined 1 group and closely mirrored the Pacific and Intermountain West phylogroups, samples from the southern Great Plains and the Southwest defined another group (similar to the South phylogroup), and a 3rd group was defined by all samples east of the Mississippi, except for samples from the New England area, which formed their own group. We found moderate to high levels of differentiation (pairwise Φ_{ST}) among all pairs of phylogroups (Table 2). The East phylogroup was differentiated from the other phylogroups. The Intermountain West phylogroup also was differentiated from the South phylogroup (Table 2).

The combination of haplotype and nucleotide diversity patterns present in each phylogroup provides some indication of demographic processes (Avise 2000). The low haplotype and nucleotide diversity in the cytochrome-*b* data for the Pacific phylogroup suggest that skunks in California were recently isolated from the their ancestral haplogroup, the Intermountain West, after animals expanded up the western side of the Sierra Nevada range (Table 3). The combination of high haplotype diversity and low nucleotide diversity in the remaining phylogroups suggests that these areas were independently colonized and isolated from other populations during the late Pleistocene. The combination of high haplotype diversity and high nucleotide diversity for the pooled data set suggests that a large amount of gene flow and admixture occurred throughout North America.

For the entire data set, Fu's F_S revealed a signature of expansion (Fu's $F_S = -6.403$, $P << 0.001$); the South phylogroup, and the East and Intermountain West phylogroups all displayed significant signatures of population expansion (Fu's $F_S = -7.083$, $P = 0.001$; Fu's $F_S = -4.319$, $P = 0.008$; Fu's $F_S = -2.748$, $P = 0.035$, respectively), whereas the Pacific phylogroup did not (Fu's $F_S = 1.137$, $P = 0.388$). These values indicated a steady expansion of striped skunks throughout much of North America. The combination of divergence estimates, phylogenetic patterns, and expansion estimates reveals the historical pattern of regional expansion within some populations (Fig. 3).

Contemporary striped skunk population structure.—Analysis of microsatellite markers indicated that none of the loci exhibited linkage disequilibrium ($P > 0.05$ after Bonferroni correction). Mean allelic richness and heterozygosity were similar for all 3 populations (Table 4). We found heterozygote deficiency at all 8 loci in the Northwest population, at 5 loci in the South population, and at 7 loci in the East population (Table 4). A Wilcoxon sign-rank test in program BOTTLENECK 1.2.02 under the 2-phase mutation model was significant for the Northwest, suggesting a past population reduction and subsequent expansion; when all populations were combined, the bottleneck signature was only marginally significant (Table 4). The Bayesian clustering algorithm using microsatellite markers in STRUCTURE indicated 3 contemporary populations of striped skunks in North America, which we define as: Northwest: samples from California, Oregon, Nevada, Wyoming, Montana, Nebraska, and North Dakota; South: samples from Arizona, Louisiana, New Mexico, and Texas; and East: samples from Georgia, Illinois, Indiana, Maine, Michigan, Ohio, Vermont, Virginia, and West Virginia. The global F_{ST} was 0.029 ± 0.005, which indicated that there was only a modest degree of divergence among populations of

TABLE 2.—Pairwise Φ_{ST} estimates based on cytochrome-*b* sequence data from 4 haplogroups determined using median-joining networks (left side of table), and pairwise F_{ST} estimates based on microsatellite markers for 3 contemporary striped skunk populations (right side of table). Based on sequential Bonferroni correction with $\alpha = 0.05$, all 4 phylogroups and all 3 contemporary populations were significantly differentiated from one another using both sets of molecular markers, suggesting strong population structure throughout North America.

Phylogroup	Pacific	Intermountain West	South	East	South	East	Northwest	Population
Pacific	—						—	Northwest
Intermountain West	0.62	—				—	0.03	East
South	0.69	0.75	—		—	0.03	0.02	South
East	0.82	0.85	0.82	—				

Table 3.—Haplotype and nucleotide diversities for the cytochrome-*b* sequences of 601 base pairs for each of the 4 striped skunk phylogroups, and for all groups combined. Only the South and East phylogroups exhibited haplotype sharing, with 3 haplotypes being present in both phylogroups.

Phylogroup	*n*	Haplotypes	Haplotype diversity	Nucleotide diversity
Pacific	11	2	0.182 ± 0.021	0.001 ± 0.001
Intermountain West	64	11	0.826 ± 0.001	0.003 ± 0.002
South	117	20	0.810 ± 0.001	0.004 ± 0.003
East	77	16	0.830 ± 0.001	0.004 ± 0.003
All specimens	269	46	0.938 ± 0.006	0.015 ± 0.008

striped skunks across different regions of North America. Pairwise F_{ST} values among the 3 populations (Table 2) indicated a modest but statistically significant amount of differentiation among populations throughout North America. Effective populations sizes (θ; Table 4) for striped skunk populations in the Northwest, South, and East were not significantly different among populations.

We also examined fine-scale population structure in the western, central, and eastern regions of the United States. Within the Northwest population, samples on either side of the Sierra Nevada (California versus Nevada and Oregon) that had divergent haplotypic signatures, all comprised a single population ($K = 1$) when microsatellite markers were analyzed in STRUCTURE. The pairwise F_{ST} between populations on either side of the Sierra Nevada range was 0.04 ($P = 0.02$). Clinal analysis within the East population showed a slight gradient in individual *q*-value with latitude near the Mississippi River; however, linear regression indicated that this cline was not significant ($r^2 = 0.56$; $F_{1,4} = 4.96$, $P = 0.09$; Fig. 4), and a pairwise $F_{ST} = 0.018$ ($P = 0.01$) between samples from Illinois, Indiana, Ohio, and Michigan and samples from Virginia, West Virginia, and Georgia suggested that they operate as a single population. Clinal analysis in the Great Plains using samples from the Northwest and South populations revealed a gradient in individual *q*-values with latitude, suggesting a pattern of secondary contact between 2 divergent lineages from north to south in the Great Plains; latitude was a significant predictor of *q*-value in the Great Plains ($r^2 = 0.80$, $F_{1,5} = 11.33$, $P = 0.02$; Fig. 4).

Discussion

During the Pleistocene, fluctuating climates and ecosystems redistributed the majority of the North American biota, which had dramatic effects on the evolutionary history and distribution of many species. Generalist species such as the striped skunk (Rosatte 1987), however, were able to persist in a variety of environmental conditions, and colonized previously unoccupied areas of the continent during glacial maxima. Unhindered by fluctuating ecosystems, the population size of striped skunks appeared to remain constant throughout the climatic fluctuations of the late Pleistocene until populations increased during the Wisconsinan glaciation (110,000-10,000 years ago). Examination of data from our study suggests that the biogeographic patterns of striped skunks represent multiple Pleistocene expansions (Fig. 3) from the ancestral clade in the south-central United States and Mexico, from which all other lineages of striped skunks descended. From Irvingtonian deposits (1.8 mya to 0.5 mya), fossil remains have been found from Florida, Colorado, and Arkansas (Anderson 2004) and suggest that striped skunks expanded across the Mississippi River and into the southeastern United States. Examination of our data suggests that the

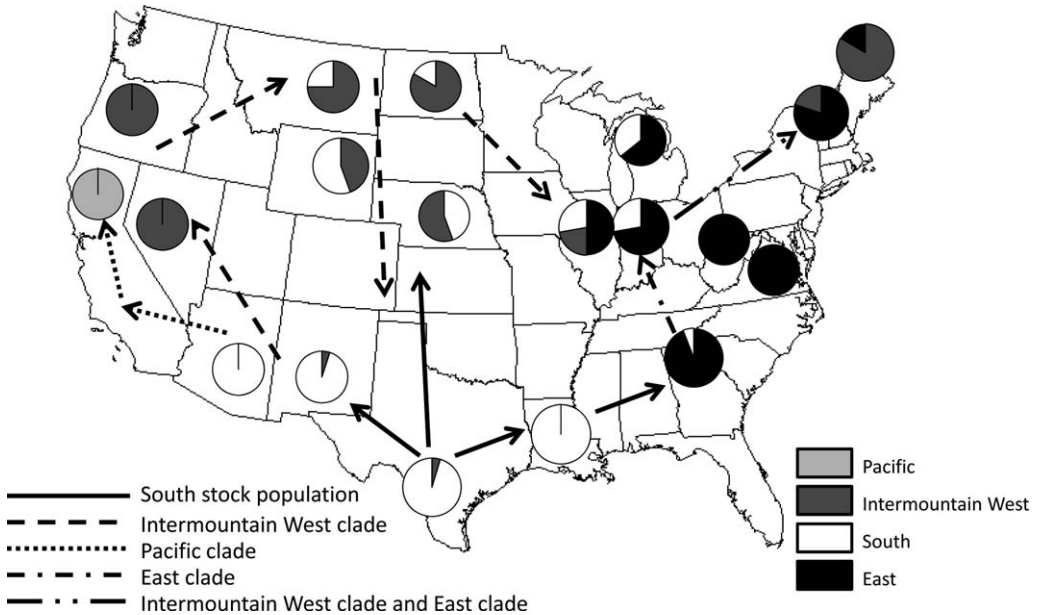

Fig. 3.—Geographic distribution striped skunk phylogroups based on 601 base pairs of cytochrome-*b* gene in mitochondrial DNA. Pie charts indicate the proportional representation of groups in each state. The hypothesized Pleistocene and Holocene dispersal patterns for striped skunk phylogroups are indicated by unique dash marks.

Table 4.—Population genetic analyses of each of the 3 striped skunk populations, for pooled data, and for each microsatellite locus. All populations had a similar effective population size (θ), and similar levels of observed and expected heterozygosity (H_O and H_E, respectively) and allelic richness (AR). One of the populations showed the presence of a population bottleneck, and the pooled data showed a marginally significant bottleneck signature. All 8 loci were out of Hardy–Weinberg equilibrium (HWE) when all samples were pooled.

	AR	H_O	H_E	θ	Bottleneck	HWE
Population						
Northwest	14.63 ± 0.39	0.75 ± 0.02	0.88 ± 0.01	4.36	$P = 0.01$	—
South	15.87 ± 0.24	0.81 ± 0.02	0.88 ± 0.02	5.36	$P = 0.25$	—
East	14.30 ± 0.30	0.74 ± 0.01	0.88 ± 0.01	3.03	$P = 0.07$	—
Pooled data	12.88 ± 0.43	0.764 ± 0.01	0.896 ± 0.01	—	$P = 0.05$	—
Microsatellite locus						
22-70	23.00	0.756	0.933	—	—	$P << 0.001$
22-67	20.00	0.789	0.888	—	—	$P << 0.001$
22-14	18.00	0.759	0.910	—	—	$P << 0.001$
42-26	17.00	0.736	0.916	—	—	$P << 0.001$
42-15	18.00	0.699	0.881	—	—	$P << 0.001$
42-25	22.00	0.843	0.889	—	—	$P = 0.02$
22-19	17.00	0.789	0.886	—	—	$P << 0.001$
42-73	15.00	0.742	0.855	—	—	$P << 0.001$

southeastern colonizers split from the ancestral population in the south-central United States and Mexico (Table 1) during the late Pleistocene when repeated glacial melting widened the Mississippi River and acted as a barrier to admixture between these 2 biogeographic regions. Ultimately, this vicariant event led to the generation of 2 separate lineages of striped skunks, 1 represented by the ancestral South phylogroup and 1 represented by the East phylogroup (see Van Gelder [1959] for a similar pattern in spotted skunks [*Spilogale*]).

The South and East populations represent the ancestral lineages from which all other lineages descended. The pattern of restricted gene flow with isolation by distance in the South phylogroup revealed by nested clade analysis indicates that stable population size and equilibrium conditions persisted throughout the late Pleistocene in this region. This population was likely stable during the oscillating ice ages due to its distance from the effects of glaciers to the north. Fu's F_S estimate for the South phylogroup indicated a signature of

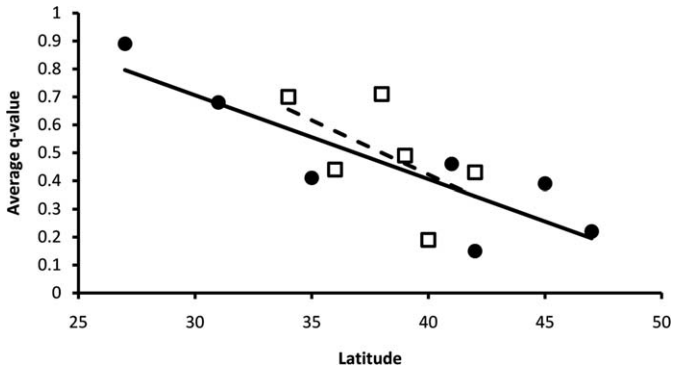

Fig. 4.—Linear regression of average *q*-value against latitude for the South and Northwest populations (black circles), and for the East population (open squares). Linear regression was significant for the South and Northwest populations ($r^2 = 0.80$; $P = 0.02$), but not for the East population ($r^2 = 0.56$; $P = 0.09$), indicating incomplete admixture in the Great Plains.

population expansion, providing more evidence that this group was the population of origin for Pleistocene and Holocene expansion across North America. The presence of allopatric fragmentation in the nested clade analysis for the East phylogroup (Fig. 1) suggests that subsequent to the South–East phylogroup split, the East phylogroup was small and isolated from western and central skunk populations for the remainder of the Pleistocene.

The retreat of the Wisconsinan glacier in the east precipitated the development of habitable ecosystems in the northeast, enabling animals in the southeast to expand north up to the Great Lakes. The complete retreat of the Laurentide ice sheet ultimately provided habitat for striped skunks in the New England regions of the United States by the middle to late Holocene. The appearance of fossil remains from that time period (8,500 years ago to 4,500 years ago) in Tennessee, Kentucky, Ohio, Pennsylvania, and New York support the hypothesis that striped skunks expanded northward toward the Great Lakes and New England throughout the Holocene; by the late Holocene (~4,500 years ago), striped skunks had expanded into much of their modern distribution (FAUNMAP Working Group 1994). Yet, we found no evidence that animals in the southeast, represented by the East phylogroup, expanded westward across the Mississippi River at any point following the initial South–East phylogroup split, which suggests that the Mississippi River was a historical barrier, and continues to be a modern barrier to dispersal by striped skunks. Indeed, examination of our microsatellite data revealed the highest levels of contemporary differentiation on either side of the Mississippi River (Table 2), suggesting that the modern populations that descended from divergent lineages have not admixed. Only in the northern half of the Mississippi River do we find evidence that animals from both the South phylogroup and Intermountain West phylogroup crossed the Mississippi River (Fig. 3).

The Mississippi River thus represents a considerable biogeographic barrier in the eastern United States; this pattern

is revealed by another habitat generalist, the raccoon. It appears that distinct lineages of raccoons persist on either side of the Mississippi, with minimal admixture between the 2 populations (Cullingham et al. 2008). In addition, phylogeographic patterns for other mammals (northern short-tailed shrew—Brant and Orti 2003), reptiles (five-lined skink [Howes et al. 2006] and black rat snake [*Elaphe obsolete*—Burbrink et al. 2000]), and amphibians (northern leopard frog [*Rana pipiens*—Hoffman and Blouin 2004] and tiger salamander [*Ambystoma tigrinum*—Templeton et al. 1995]) all indicate that the Mississippi River was a major biogeographic barrier, responsible for vicariance among lineages, and ultimately generated distinct phylogroups within species. In some species, it appears that the Mississippi River also is structuring contemporary populations.

The late Pleistocene was not only a time of expansion to the east; striped skunks also expanded westward during this time. Consensus estimates of divergence indicated that the Intermountain West phylogroup diverged from the South phylogroup about 200,000 years ago during the Illinoian glaciation (Table 1). We infer, based on patterns of haplotypic distribution and the presence of inhospitable habitat to the north, that striped skunks colonized the Great Basin from a southerly route. As extensive montane glaciers formed in the Rocky Mountains during the Illinoian glacial maximum, the South and Intermountain West phylogroups diverged. Subsequent phylogroup formation occurred once the far west was colonized by striped skunks and then were isolated from the Great Basin population (the Intermountain West phylogroup) by the Sierra Nevada range. The resulting isolation of populations to the west of the Sierra Nevada formed the Pacific phylogroup, which split from the Intermountain West phylogroup approximately 132,000 years ago during the Sangamonian interglacial stage (Table 1).

Fossil remains dated to the Wisconsinan stage (70,000 years ago to 10,000 years ago) from Idaho and central California suggest that striped skunks expanded north along both sides of the Sierra Nevada. During the Holocene warming trend, however, it appears that these isolated populations may have once again become admixed. Microsatellite markers suggest admixture of populations on either side of the Sierra Nevada range such that they are not distinguishable from one another. Thus, the signature of Sangamonian vicariance observed in the maternally inherited mitochondrial genome is obscured by contemporary gene flow as observed in the nuclear genome. This anomalous pattern of spatial partitioning could be influenced by male-biased dispersal in this carnivore species (Sargeant et al. 1982). Nonetheless, it appears that these 2 western populations were separated during the last interglacial period and have likely been admixing since the late Wisconsinan or early Holocene; however, a more detailed biogeographic study of striped skunks in the west is warranted.

The warming trend following the end of the Pleistocene led to the retreat of high-elevation glaciers in the Rocky Mountains, and opened up high-latitude colonization routes for striped skunks. The distribution of Intermountain West haplotypes from the Great Basin to the northern Great Plains suggests that during the late Pleistocene or early Holocene, individuals in the Great Basin crossed the Continental Divide to the north, and then expanded east and south into the northern and central Great Plains. As the Great Plains developed into a mixture of grassland, parkland, and forest ecosystems (Pielou 1991), the Great Plains also became amenable (Bixler and Gittleman 2000) to the population in the southern Great Plains and allowed them to expand northward. Eventually, colonizers from the northern and southern Great Plains admixed such that the South phylogroup and Intermountain West phylogroup came into contact in the central Great Plains. The secondary contact of these lineages has led to a clinal pattern of microsatellite genotypes (Fig. 4), suggesting that the Great Plains is currently in a dynamic phase of nonequilibrium as 2 populations that remained separated for 150,000 years have recently come into contact. Middle Holocene (~8,500 years ago) fossils that appear in Nebraska and Kansas and late Holocene fossils (~5,000 years ago) from several states (i.e., Minnesota, Iowa, Nebraska, Kansas, and Oklahoma) support the expansion of striped skunks across the Great Plains throughout the Holocene (FAUNMAP Working Group 1994).

Contemporary demographic processes also shape the patterns of genetic diversity and structure in striped skunks. The signal of a population bottleneck found in the microsatellite data from the Northwest population at 1st appears to contradict the results of the mitochondrial data, which indicated steady population expansion of striped skunks throughout North America. However, striped skunks are reservoirs for many diseases including rabies (Blanton et al. 2009), canine distemper (Gehrt et al. 2010), and tularemia (Berrada et al. 2006). California and the Great Plains are the foci of 3 strains of rabies for which striped skunks are the major disease reservoir (Blanton et al. 2009; Crawford-Miksza et al. 1999). Rabies is virtually always fatal, and routinely decimates populations of striped skunk where the disease is endemic, leading to periodic fluctuations in population size (Gehrt et al. 2006), the signature of which was likely captured by the bottleneck test. The East population, on the other hand, does not have an endemic strain of skunk rabies (although striped skunks succumb to the raccoon strain in the east—Blanton et al. 2009) and in this population we found no evidence of a population bottleneck.

Our reconstruction of late Pleistocene, Holocene, and recent population structure and demography provide the phylogenetic context needed to evaluate the importance of dispersal routes and biodiversity hot spots for North American generalist species. Western mountain ranges and major river drainages also have been implicated as biogeographic barriers promoting isolation of populations during the Pleistocene for the deer mouse and raccoon (Cullingham et al. 2008; Dragoo et al. 2006; Yang and Kenagy 2009). By the end of the Pleistocene, deer mice appear to have used the same southern colonization route to expand from New Mexico into Texas (Dragoo et al. 2006) that striped skunks used during the Illinoian glacial stage to expand westward. The Sierra Nevada range also structured western populations of the deer mouse. Population

structure of the deer mouse mirrors populations of striped skunks and suggests that the Sierra Nevada was a source of vicariance for multiple species, and provided separate northward colonization routes on either side of the mountain range (Yang and Kenagy 2009). Additionally, genetic diversity of the deer mouse on a continental scale is similar to diversity patterns of the striped skunk in the Great Plains and upper Midwest with high levels of admixture of separate lineages. For multiple generalist species, it appears that midcontinental North America represents hot spots of intraspecific biodiversity where secondary contact of divergent lineages has led to high levels of genetic diversity and the admixture of previously separated lineages (Blackburn and Measey 2009; He et al. 2008; Hopper and Gioia 2004; Petit et al. 2003).

The comparative phylogeography among deer mice, raccoons, and striped skunks implies that multiple generalist mammalian species shared similar colonization and vicariance events, which resulted in similar contemporary patterns of population structure. The presence of multiple ancient lineages in contemporary populations of deer mice, raccoons, and striped skunks suggests that it may be worthwhile to further characterize modern gene flow and secondary contact among divergent lineages of these and other generalist species. Generalist mammals are often zoonotic disease reservoirs (Cullingham et al. 2008; Dragoo et al. 2006; Ngamprasertwong et al. 2008) that can respond rapidly to habitat alteration. When distantly related lineages admix, so can their pathogens, which increases the potential for genetic recombination in pathogens, and the introduction of novel pathogens into naïve populations (Brooks and Hoberg 2007; Campos-Krauer and Wisely 2011; Hoberg and Brooks 2008; Holmes 2004). By investigating the phylogeography of host species, we can begin to examine host–pathogen coevolution and the potential for disease emergence or pandemics in new habitats.

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APPENDIX I

List of samples of *Mephitis mephitis* used in the present study, including: state, identification number, county, GenBank accession number, and sex. Samples were obtained as described in ''Materials and Methods.''

Arizona.—AZ004, Cochise, JN008613, female; AZ007, Cochise, JN008636, male; AZ008, Cochise, JN008597, female.

California.—CA02, Sacramento, JN008604, male; CA03, Sacramento, JN008534, male; CA04, Sacramento, JN008527, male; CA05, Sacramento, JN008573, male; CA07, Humboldt, JN008529, unknown; CA08, Humboldt, JN008574, unknown; CA09, Humboldt, JN008633, unknown; CA16, Butte, JN008572, male; CA17, Butte, JN008593, female; CA18, Butte, JN008483, male; CA19, Butte, JN008612, male.

Georgia.—C11, Walker, JN008470, female; C34, Walker, JN008665, female; E1, Walker, JN008652, female; E2, Walker, JN008606, male; E3, Catoosa, JN008464, female; E4, Walker, JN008605, unknown; E5, Whitfield, JN008486, unknown; E6, Whitfield, JN008482, unknown; E7, Walker, JN008504, unknown; E8, Walker, JN008507, unknown; GA9005, Catoosa, JN008476, male; GA9006, Catoosa, JN008697, female; GA9007, Walker, JN008584, male; GA9008, Walker, JN008648, male; GA9011, Walker, JN008521, male; GA9013, Chattooga, JN008675, male; GA9025, Walker, JN008459, female.

Illinois.—IL001, Madison, JN008595, male; IL002, Crawford, JN008497, female; IL003, Effingham, JN008703, male; IL004, Crawford, JN008647, female; IL005, Clark, JN008676, male; IL006, Jasper, JN008503, male; IL007, Crawford, JN008680, male; IL008, Vermillion, JN008693, male; IL009, Crawford, JN008585, male; IL010, Crawford, JN008582, male; IL011, Crawford, JN008614, male; IL012, Jasper, JN008502, male; IL013, Jersey, JN008685, female; IL014, DuPage, JN008533, male; IL015, DuPage, JN008611, female; IL016, Cook, JN008598, female; IL018, Sangamon, JN008663, male; IL019, Lake, JN008640, male.

Indiana.—IN003, Carroll, JN008700, female; IN004, Harrison, JN008460, male; IN006, Harrison, JN008477, female; IN007, Harrison, JN008489, female; IN008, Harrison, JN008629, male; IN015, Steuben, JN008500, male; IN017, Steuben, JN008508, male; IN018, Tippecanoe, JN008596, male; IN019, Montgomery, JN008631, unknown; IN020, Montgomery, JN008469, unknown; IN021, Clark, JN008626, male; IN022, Daviess, JN008627, unknown; IN023, Pike, JN008480, unknown; IN024, Pike, JN008610, unknown; IN025, LeGrange, JN008632, unknown; IN026, Noble, JN008515, unknown.

Louisiana.—LA001, Caldwell, JN008615, male; LA002, Ouachita, JN008499, male; LA003, Franklin, JN008468, male; LA004, JN008639, Ouachita, female; LA005, Ouachita, JN008461, female; LA006, Tensas, JN008447, male; LA007, Ouachita, JN008656, male;

LA008, Madison, JN008520, male; LA009, Madison, JN008570, male; LA010, Madison, JN008641, male; LA011, Madison, JN008454, female; LA012, Madison, JN008463, male; LA014, Madison, JN008511, male; LA015, Caldwell, JN008541, female; LA016, Madison, JN008455, male; LA017, Franklin, JN008495, male; LA018, Ouachita, JN008580, male; LA019, Ouachita, JN008474, male; LA020, Madison, JN008537, male.

Maine.—ME478, Aroostook, JN008531, female; ME479, Penobscot, JN008698, male; ME480, Penobscot, JN008667, female; ME481, Washington, JN008525, female; ME486, Penobscot, JN008696, female; ME489, Aroostook, JN008655, male; ME492, Aroostook, JN008557, male; ME494, Aroostook, JN008642, male; ME496, Aroostook, JN008635, female; ME497, Penobscot, JN008646, unknown; ME498, Aroostook, JN008510, male; ME499, Penobscot, JN008601, female; ME510, Penobscot, JN008654, male; ME511, Aroostook, JN008577, male; ME512, Aroostook, JN008638, female; ME513, Aroostook, JN008576, male.

Michigan.—MI001, Ingham, JN008488, male; MI002, Shiawassee, JN008692, male; MI003, Ingham, JN008600, male; MI004, Ingham, JN008514, male; MI005, Gratiot, JN008591, male; MI006, Ingham, JN008539, male; MI007, Clinton, JN008602, unknown; MI008, Clinton, JN008492, male; MI009, Clinton, JN008704, unknown; MI010, Clinton, JN008690, male; MI011, Clinton, JN008583, male; MI012, Clinton, JN008479, male; MI013, Clinton, JN008688, male; MI014, Clinton, JN008609, male.

Montana.—MT001, Yellowstone, JN008699, male; MT002, Rosebud, JN008709, male; MT003, Rosebud, JN008490, female; MT004, Rosebud, JN008686, male; MT005, Musselshell, JN008538, male; MT006, Rosebud, JN008608, male; MT007, Rosebud, JN008702, male; MT008, Rosebud, JN008630, female; MT009, Rosebud, JN008562, male; MT010, Rosebud, JN008548, male; MT011, Rosebud, JN008607, female; MT012, Dawson, JN008506, male; MT013, Rosebud, JN008586, female; MT014, Rosebud, JN008691, male; MT015, Rosebud, JN008659, male; MT017, Yellowstone, JN008694, male.

Nebraska.—NE01, Fillmore, JN008587, unknown; NE03, Douglas, JN008517, female; NE04, Cass, JN008524, male; NE05, York, JN008657, male; NE06, Hall, JN008588, unknown; NE09, Seward, JN008522, male; NE010, Fillmore, JN008571, male; NE011, Nuckolls, JN008637, unknown; NE013, Nuckolls, JN008677, male.

Nevada.—NV002, Washoe, JN008575, male; NV003, Washoe, JN008625, male; NV004, Washoe, JN008619, male; NV005, Washoe, JN008618, male.

New Mexico.—NM001, Dona Ana, JN008556, unknown; NM002, Dona Ana, JN008551, male; NM003, Dona Ana, JN008569, female; NM004, Dona Ana, JN008599, male; NM005, Dona Ana, JN008446, female; NM006, Dona Ana, JN008559, female; NM007, Dona Ana, JN008549, male; NM008, Dona Ana, JN008535, male; NM009, Dona Ana, JN008547, female; NM010, Dona Ana, JN008701, male; NM011, Dona Ana, JN008471, male; NM012, Dona Ana, JN008617, male; NM013, Dona Ana, JN008453, female; NM014, Dona Ana, JN008540, female; NM015, Dona Ana, JN008452, female; NM016, Dona Ana, JN008565, female; NM017, Dona Ana, JN008679, female; NM018, Dona Ana, JN008472, male; NM019, Dona Ana, JN008594, male; NM020, Dona Ana, JN008592, male.

North Dakota.—ND002, Slope, JN008674, male; ND005, Ransom, JN008567, male; ND006, Ransom, JN008590, male; ND007, Logan, JN008475, male; ND011, McHenry, JN008558, male; ND012, McHenry, JN008651, female.

Ohio.—IN010, Williams, JN008684, male; IN013, Williams, JN008707, male.

Oregon.—OR001, Wasco, JN008481, male; OR002, Crook, JN008473, male; OR003, Deschutes, JN008494, male; OR004, Crook, JN008683, female; OR005, Deschutes, JN008650, female; OR006, Deschutes, JN008462, male; OR007, Deschutes, JN008466, female; OR008, Crook, JN008589, male; OR010, Crook, JN008465, female.

Texas.—TX001, Angelina, JN008536, unknown; TX002, Brown, JN008552, unknown; TX003, Webb, JN008568, unknown; TX004, Webb, JN008554, unknown; TX005, Webb, JN008530, unknown; TX006, Webb, JN008581, unknown; TX008, Rusk, JN008544, unknown; TX009, Brown, JN008512, unknown; TX010, Comanche, JN008501, unknown; TX011, Comanche, JN008550, unknown; TX012, Hamilton, JN008579, unknown; TX013, Rusk, JN008578, unknown; TX014, Comanche, JN008653, unknown; TX015, Brown, JN008526, unknown; TX016, Hidalgo, JN008566, unknown; TX017, Rusk, JN008669, unknown; TX018, Comanche, JN008491, unknown; TX019, Comanche, JN008445, unknown; TX020, Brown, JN008644, unknown; TX021, Angelina, JN008670, unknown; TX022, Angelina, JN008689, unknown; TX023, Moore, JN008518, unknown; TX024, Cherokee, JN008542, unknown; TX028, Collin, JN008532, unknown; TX029, Tarrant, JN008616, unknown; TX030, Wichita, JN008678, unknown; TX031, Tarrant, JN008509, unknown; TX032, Midland, JN008441, unknown; TX033, Wichita, JN008496, unknown; TX035, Collin, JN008444, unknown; TX036, Hutchinson, JN008603, unknown; TX037, Wichita, JN008498, unknown; TX038, Wichita, JN008484, unknown; TX039, Cherokee, JN008563, unknown; TX040, Gregg, JN008545, unknown; TX041, Wichita, JN008553, unknown; TX042, Collin, JN008505, unknown; TX043, Gregg, JN008442, unknown; TX044, Collin, JN008485, unknown; TX045, Wichita, JN008561, unknown; TX046, Johnson, JN008555, unknown; TX047, Midland, JN008443, unknown; TX049, Hutchinson, JN008543, unknown.

Vermont.—VT0901, Orleans, JN008519, male; VT0902, Franklin, JN008493, male; VT0903, Franklin, JN008564, male; VT0905, Orleans, JN008456, female; VT0907, Lamoille, JN008664, female.

Virginia.—VA001, Washington, JN008661, male; VA002, Washington, JN008660, male; VA003, Scott, JN008634, male; VA004, Washington, JN008516, male; VA005, Washington, JN008643, male.

West Virginia.—WV1, Cabell, JN008671, unknown; WV2, Mason, JN008457, unknown; WV3, Mason, JN008708, unknown; WV4, Wayne, JN008662, unknown; WV5, Randolph, JN008673, male; WV6, Randolph, JN008687, female; WV7, Mason, JN008682, male; WV8, Mason, JN008458, male; WV9, Jackson, JN008672, male; WV11, Monongalia, JN008467, male; WV12, Wetzel, JN008705, male; WV13, Harrison, JN008695, male; WV14, Preston, JN008649, male; WV15, Marshall, JN008658, male; WV17, Marion, JN008523, male; WV18, Upshur, JN008478, male; WV19, Taylor, JN008666, male; WV20, Barbour, JN008560, unknown.

Wyoming.—WY001, Crook, JN008628, male; WY002, Fremont, JN008451, unknown; WY003, Fremont, JN008487, unknown; WY004, Fremont, JN008622, unknown; WY005, Fremont, JN008448, unknown; WY006, Fremont, JN008668, unknown; WY007, Fremont, JN008450, unknown; WY008, Fremont, JN008546, unknown; WY009, Fremont, JN008449, unknown; WY010, Fremont, JN008624, unknown; WY011, Fremont, JN008623, unknown; WY012, Fremont, JN008681, unknown; WY013, Fremont, JN008513, unknown; WY014, Natrona, JN008528, unknown; WY015, Natrona, JN008706, male; WY016, Natrona, JN008621, male; WY019, Carbon, JN008645, unknown; WY021, Carbon, JN008620, male.