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THE COMBINED EFFECTS OF RIVERS AND REFUGIA GENERATE EXTREME CRYPTIC FRAGMENTATION WITHIN THE COMMON GROUND SKINK (*SCINCELLA LATERALIS*)

Nathan D. Jackson[1,2] and Christopher C. Austin[1,3]

[1]*Museum of Natural Science and Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803*

[2]*E-mail: njacks4@lsu.edu*

[3]*E-mail: ccaustin@lsu.edu*

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Rivers can act as both islands of mesic refugia for terrestrial organisms during times of aridification and barriers to gene flow, though evidence for long-term isolation by rivers is mixed. Understanding the extent to which riverine barrier effects can be heightened for populations trapped in mesic refugia can help explain maintenance and generation of diversity in the face of Pleistocene climate change. Herein, we implement phylogenetic and population genetic approaches to investigate the phylogeographic structure and history of the ground skink, *Scincella lateralis*, using mtDNA and eight nuclear loci. We then test several predictions of a river–refugia model of diversification. We recover 14 well-resolved mtDNA lineages distributed east–west along the Gulf Coast with a subset of lineages extending northward. In contrast, ncDNA exhibits limited phylogenetic structure or congruence among loci. However, multilocus population structure is broadly congruent with mtDNA patterns and suggests that deep coalescence rather than differential gene flow is responsible for mtDNA–ncDNA discordance. The observed patterns suggest that most lineages originated from population vicariance due to riverine barriers strengthened during the Plio–Pleistocene by a climate-induced coastal distribution. Diversification due to rivers is likely a special case, contingent upon other environmental or biological factors that reinforce riverine barrier effects.

KEY WORDS: **Incomplete lineage sorting, lizard, phylogeography, Pleistocene, riverine barrier hypothesis, southeastern North America.**

The evolutionary legacy imparted by Pleistocene climate change to naturally dispersing populations and species has been subject to considerable investigation (Smith 1957; Blair 1958; Lundelius et al. 1983; Hewitt 1996, 2000; Provan and Bennett 2008), in part due to what this legacy can tell us about the climatic conditions experienced during the Quaternary as well as about the processes of species formation and extinction (Waltari et al. 2007). Recent reviews of phylogeographic studies implemented across several continents (Brunsfeld et al. 2001; Hewitt 2004; Soltis et al. 2006; Gomez and Lunt 2007; Byrne 2008; Stork and Turton 2008; Zeisset and Beebee 2008) reveal remarkable heterogeneity in the population genetic responses of codistributed species to the Pleistocene, reflecting that climate change is not a deterministic driver of evolution but just one among many factors (e.g., ecological, genetic, geographical) that interact to shape population genetic patterns (Byrne 2008). Climatic fluctuations throughout the Pleistocene resulted in a variety of both diversifying (e.g., Lister 2004; Provan and Bennett 2008) and homogenizing (e.g.,

Zink et al. 2004; Loehle 2007) consequences depending on the taxa assayed, and understanding the interaction of climate change with other forces can help explain the distinctive evolutionary histories observed for species that have experienced the same climatic cycles.

Rivers are one force that may interact with Pleistocene-induced range-shifting to maintain and even generate diversity in the face of reduced, consolidated, or extirpated populations (Haffer 1997; Schneider et al. 1999; Anthony et al. 2007). For many terrestrial organisms, rivers (and their surrounding regions) can act both as refugial mesic habitat in the face of xerification (Delcourt and Delcourt 1981; Haffer 1997) and as barriers to gene flow between populations on either side (Sick 1967). The refugial effect of rivers can both sequester pre-Pleistocene diversity that would otherwise not survive in the wider more arid environments (Evans et al. 2004) and generate new diversity by isolating previously interbreeding populations to refugial islands (Holder et al. 1999). This effect is however contingent upon the existence of such refugia and the ability of a species to reach and survive in potentially small, ephemeral, and isolated pockets of suboptimal habitat (Davis 1983). The barrier effect of rivers, likely strongest far from the headwaters where they are widest and most impenetrable to would-be migrants (Haffer 1992), can potentially generate or retain population genetic structure within a single refugium. Climate change can also strengthen the barrier effect of a river if wide-ranging populations normally able to circum-navigate a riverine barrier at the headwaters are forced to contract their ranges toward warmer, moister habitat near the mouth of a river where it is most isolating (Capparella 1991; Haffer 1997). This river–refugia effect is contingent upon the inability of organisms to migrate across wide rivers and the persistence of populations in multiple refugia.

The southeastern United States has been the focus of substantial phylogeographic inquiry over the past few decades (Auffenberg and Milstead 1965; Walker and Avise 1998; Avise 2000; Soltis et al. 2006), with much attention being paid to the identification of both geographic barriers to gene flow and hospitable mesic refugia that might have retained or produced its substantial diversity during glacial maxima (Delcourt 2002; Waltari et al. 2007). Much of this attention has highlighted the evolutionary importance of two major rivers in the region: the Mississippi and Apalachicola Rivers. Data from both phylogeographic inquiry (reviewed in Soltis et al. 2006) as well as pollen and macro-fossils (Delcourt and Delcourt 1981; Watts 1983; Jackson et al. 2000) provide strong evidence for the existence of many warm mesic-adapted species within the Mississippi River Valley and Apalachicola River-region of northern Florida throughout the Pleistocene. Additionally, well-established genetic discontinuities within a variety of taxa are concordant with the Mississippi (e.g., Brant and Orti 2002; Leaché and Reeder 2002), Apalachicola (e.g., Burbrink et al. 2000; Pauly et al. 2007), and to a lesser extent, Tombigbee Rivers (e.g., Gill et al. 1993; Gamble et al. 2008), indicating an important role for these rivers in driving diversification in the region.

Although additional Pleistocene refugia in high-elevation regions such as the Ouachita (Shepard and Burbrink 2008), Ozark (Austin et al. 2004), and Appalachian mountains (Church et al. 2003; Kozak et al. 2006; Walker et al. 2009) must be invoked to explain extant phylogeographical patterns further north, identification of other refugia along the Gulf Coast (which largely lacks the topography allowing for mesic refugia in highlands) has been more difficult. Pockets of mesic refugia likely existed throughout the Gulf Coast (Davis 1983), particularly along the bluffs of major rivers (Delcourt 2002), but it is unclear how extensive or reliable such refugia were during the Pleistocene (Jackson et al. 2000). Additionally, beyond the Apalachicola, Mississippi, and Tombigbee Rivers, a potential long-term barrier effect of major rivers in southeastern North America has received little support for terrestrial organisms (but see Pounds and Jackson 1981; Kozak et al. 2006; Lemmon et al. 2007) and empirical evidence is mixed on whether such smaller, although still formidable, rivers are sufficiently impenetrable or enduring to permit evolutionarily significant genetic or phenotypic divergence to accumulate between populations on opposite banks (Haffer 1969; Capparella 1991). Given the identification of such pocket refugia along the Gulf Coast, the potential for the isolating power of major rivers to be strengthened by Pleistocene-induced range contractions of populations into riverine-associated refugia can be investigated.

Here, we report on the finding of an unusual pattern of iterative mtDNA population fragmentation observed in the common ground skink (*Scincella lateralis*) that is consistent with diversification by a combination of Pleistocene refugia and rivers. These findings suggest a more consistent supply of mesic refugia along the Gulf Coast than has previously been shown for this region (Jackson et al. 2000; Loehle 2007; Gonzales et al. 2008). In this study, we describe and analyze the mtDNA pattern, assess the consistency of this pattern across the genome at eight nuclear loci, and test three predictions of a river–refuge origin of diversity (Capparella 1991; Haffer 1997): (1) Distinct lineage boundaries or genetic discontinuities should be delineated by rivers, with most rivers associated with divergence near the coast, and only the largest rivers associated with divergence further north, (2) Recent population growth of *S. lateralis* populations from hypothesized refugia should be evident, and (3) Estimates of divergence should date to or near the Plio-Pleistocene. Results from this study highlight the potential power of rivers as an evolutionary force, but also the contingency of rivers-as-barriers, their effectiveness being dependent upon other, often labile, environmental or biological factors.

Scincella lateralis, one of the most abundant reptiles in the southeastern United States (Dundee and Rossman 1989; Akin 1998; Conant and Collins 1998) is well-suited for a study on the isolating-potential of rivers and refugia due to its low vagility (Brooks 1967; Fitch and Achen 1977) and wide-ranging distribution across most major rivers along the southeastern coastal plains. Populations exhibit a similar morphology across the species range (Lewis 1951; Johnson 1953; Brooks 1967) and no subspecies or geographical variants have been proposed. Although exhibiting a wide tolerance to a variety of habitats (Milstead et al. 1950; Dundee and Rossman 1989), *S. lateralis* is reliant on warm-temperate and mesic conditions (Milstead 1960; Ashton and Ashton 1985) best provided by the present-day deciduous and mixed forests of the southeastern United States.

Materials and Methods

SAMPLING

We collected tissue from 367 ingroup specimens (Table S1) representing 100 sampling localities (Table S2) from throughout the range of the species, with some attention to sampling near major rivers (Fig. 1). Two additional samples, *S. gemmingeri* and *Sphenomorphus cherriei*, were included as outgroup specimens, in part based on previous phylogenetic analysis (Honda et al. 2003). The complete cytochrome *b* mitochondrial gene (cyt*b*), including 33 base pairs of the downstream flanking tRNA-Thr gene (1177 total base pairs), was sequenced for all individuals. A geographically representative subsample of 63 individuals (including the outgroup *S. gemmingeri*) was selected for further sequencing at eight nuclear loci (4673 total base pairs; Table S3). These loci include one intron: selenoprotein T (SELT; 852 bp); one protein-coding gene: the prolactin receptor (PRLR; 558 bp); and six noncoding genomic loci (ranging from 443 to 641 bp), discussed below (Table S4).

COLLECTION OF GENETIC MATERIAL

Either liver or tail tissue was sampled from each lizard and preserved in ≥95% ethanol and/or stored at −80°C. Genomic DNA was extracted using either salt-extraction (Fetzner 1999) or a Qiagen DNeasy extraction kit (Qiagen, Valencia, CA). Six noncoding nuclear loci were developed from select cloned sequences screened from a genomic microsatellite library created from a single *S. lateralis* individual (sample NDJ764; Table S1). Initially, of 192 sequenced clones, 20 candidate loci were selected from clones either lacking microsatellites or containing substantial microsatellite flanking regions. Primer pairs were developed for these loci using Primer 3 (Rozen and Skaletsky 2000) and then optimized for the sequencing of an initial screening set of eight individuals (representative of the major mtDNA variation as then understood for the species). Ascertainment bias was minimized by (1) using a large screening panel composed of divergent populations and (2) basing the inclusion of loci into the final dataset on our ability to obtain quality sequences rather than on the variability of observed polymorphisms (Rogers and Jorde 1996; Brumfield et al.

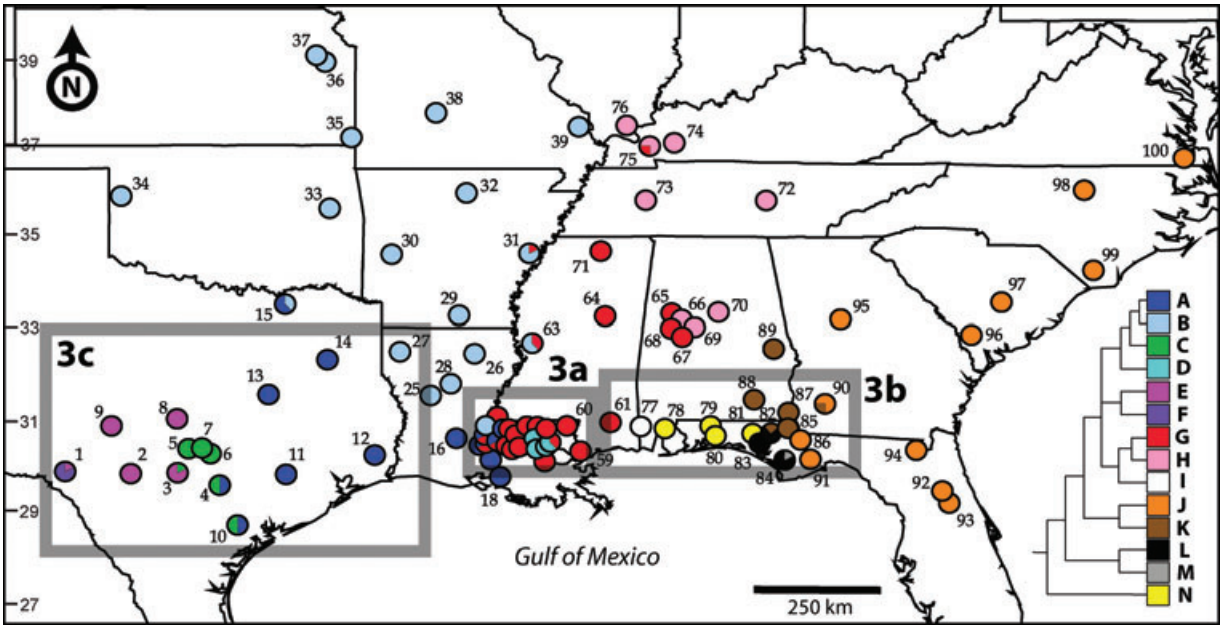

Figure 1. Geographical distribution of localities sampled for this study, with numbers corresponding to locality numbers in Table S2. Circle colors correspond to 14 reconstructed mtDNA clades, where localities harboring multiple clades are indicated by circles with mixed colors filled in proportion to clade representation. Gray boxes outline areas depicted in more detail in Figure 3A–C. Latitudinal coordinates are indicated along the border of the map.

2003). All loci were compared against sequences in an online genome database using BLASTN (Altschul et al. 1997) to assess homology with known genomic regions.

Polymerase chain reaction (PCR) of genomic DNA was carried out in accordance with standard protocols (Austin et al. 2009) and amplicons were purified by combining 5 μl PCR product with 0.25 μl Exonuclease I (20 units/μl), 0.25 μl of Antarctic phosphatase (5 units/μl), 0.25 μl 10× buffer (50 mM Bis-Tris-Propane-HCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$), and 4.25 μl purified water, followed by incubation for 20 min at 37°C and 15 min at 80°C. Double-stranded cycle-sequencing was carried out for each amplicon using a BigDye Terminator cycle-sequencing kit version 3.1 (Applied Biosystems, Foster City, CA). After sequences were cleaned using Sephadex, they were electrophoresed on a 3100 Genetic Analyzer (Applied Biosystems).

DNA ALIGNMENT, PHYLOGENETIC, AND NETWORK ANALYSIS

Sequences were edited and assembled into contigs using Sequencher version 4.6 (GeneCodes, Ann Arbor, MI) and cyt*b* and PRLR datasets were translated into amino acids to check alignment. The alignment of noncoding regions was carried out using Clustal X version 2.0 (Larkin et al. 2007) and results were adjusted as necessary by eye. For nuclear datasets, haplotype phase was reconstructed using a Markov chain Monte Carlo (MCMC) algorithm as implemented in PHASE version 2.1 (Stephens et al. 2001; Stephens and Scheet 2005). The program was run multiple times for each dataset and haplotype frequency and goodness of fit were compared across runs to assess the consistency of results. Six nuclear sequences were unreadable due to heterozygous indels and removed from the analysis.

We collapsed each dataset into unique haplotypes using Collapse version 1.2 (Posada 1999) and reconstructed gene phylogenies using both Bayesian inference (BI) and maximum likelihood (ML) optimality criteria. The most likely model of nucleotide substitution was selected (using Akaike information criterion) for each locus (and for codon-based partitions of datasets for protein-coding loci) after performing likelihood-ratio tests in Modeltest version 3.7 (Posada and Crandall 1998). For the cyt*b* dataset, we performed a full likelihood heuristic search (200 replicates) in RAxML-HPC version 7.0.3 (Stamatakis 2006) assuming a general time-reversible (GTR) model of evolution with Γ-distributed rate heterogeneity, four rate categories, and an estimation of the proportion of invariable sites. To assess nodal support, we also performed 1000 replicates of nonparametric bootstrapping implemented in RAxML-HPC version 7.0.3. BI phylogenies for cyt*b* and each nuclear locus were reconstructed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). For each dataset, searches were carried out for two independent runs each consisting of four Markov chains that were permitted to run for 5–20 million generations, with sampling every 1000 generations. Convergence was confirmed by inspecting split frequency plots constructed using AWTY (Nylander et al. 2008) and 50% consensus trees were constructed using post-burn-in genealogies. All eight nuclear datasets were concatenated and a partition homogeneity test (100 replicates) was implemented in PAUP* version 4.0b10 (Swofford 2002) to assess the level of well-supported discordance among individual topologies. Simultaneous analysis was not carried out via concatenation given obvious topological differences among gene histories (Kubatko and Degnan 2007; Edwards 2009) and the significant degree of geographical contact and migration observed among some ncDNA populations (see Results) renders these datasets poorly suited for novel methods of multilocus phylogenetic reconstruction (Brumfield et al. 2008; Eckert and Carstens 2008).

Finally, given the potential for multifurcations and reticulations within intraspecific gene trees (Rosenberg and Nordborg 2002), we also constructed haplotype networks for single-gene nuclear datasets in order to better visualize such nonbifurcating relationships (Posada and Crandall 2001). Networks were constructed using statistical parsimony (Templeton et al. 1992) as implemented in TCS version 1.21 (Clement et al. 2000) at the 95% confidence level.

POPULATION DIVERSITY AND STRUCTURE

Standard haplotype (Hd) and nucleotide (π) diversity indices were calculated for each single gene dataset and mtDNA lineage using Arlequin version 3.1.1 (Excoffier 2005). Average corrected (Kimura 2-parameter; K2P) and uncorrected pairwise genetic distances within and between major mtDNA lineages were calculated using MEGA version 3.1 (Kumar et al. 2004). Rough estimates of divergence times among mtDNA clades were calculated assuming a molecular clock that represents the minimum and maximium of the 1–2% range of cyt*b* divergence rates estimated for various small-bodied lizards and lygosomine skinks (Austin 1995; Brown and Pestano 1998; Gübitz et al. 2000; Malhotra and Thorpe 2000; Poulakakis et al. 2005). We also investigated substitution rate heterogeneity among mtDNA lineages by performing a phylogenetically weighted relative rates test in RRTREE (Robinson-Rechavi and Huchon 2000).

Population structure of the eight nuclear loci was investigated using a Bayesian clustering method implemented in Structure version 2.2.3 (Pritchard et al. 2000). We first estimated the most likely number of populations (K), as well as the most probable individual assignments to populations, given multilocus genotypic data. Applying the linkage model (Falush et al. 2003) to the raw phased sequence data (where linkage was allowed within, but not among loci), we ran Structure for all values of K between $K = 1$ and $K = 8$ ten times each for at least 150,000 generations (with an additional burn-in of 200,000). After it was determined that

$K = 3$ had the highest likelihood (see below), we also ran Structure for each of these three populations separately three times each for $K = 1$ through $K = 6$. The mean likelihood estimate [ln Pr(X | K)] from replicate runs for each value of K was used to estimate the posterior probability for each K [Pr(K | X)] by assuming a uniform prior on K (Pritchard et al. 2000).

Estimates of θ ($= 4Ne\mu$) and migration rates among the three Structure-inferred populations were obtained jointly for the ncDNA dataset by carrying out parameter searches using a Metropolis MCMC Bayesian-style sampling algorithm implemented in LAMARC version 2.1.3 (Kuhner et al. 1998; Kuhner 2006). Because the coalescent process as modeled here assumes no genealogical reticulation due to recombination, we used a dataset from which recombining regions were removed. Using the program IMgc (Woerner et al. 2007) we generated recombination-free blocks of data by filtering out all regions in the multilocus dataset that violate the four-gamete rule for recombination (Hudson and Kaplan 1985). Several independent chains were run for 5–10 million generations, with sampling every 100–140 steps, using default priors, and convergence was assessed using Tracer version 1.4 (Drummond and Rambaut 2007). To convert scaled parameter estimates to demographic estimates, we assumed a generation time of 1.7 years estimated from *S. lateralis* population age structure and survivorship data using the equation $G = \alpha + [s/(1 - s)]$ (Sæther et al. 2005), where α = the mean first year breeding age (1) and s = the adult survivor rate (0.41; Brooks 1967). We also assumed a mean nuclear clock calibrated to both 1% and 2% cytb rates by using divergence from the outgroup *S. gemmingeri* as a calibration point.

ASSOCIATION OF DIVERSITY WITH RIVERINE BARRIERS

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to compare ncDNA population structure with mtDNA phylogeny as well as assess the association of multilocus diversity with major rivers. To do this, we partitioned the concatenated ncDNA dataset in three different ways. (1) We first assessed the hierarchical allocation of genetic variation for "minor" Structure-inferred populations (depicted in Fig. 5B) nested within "major" Structure-inferred populations (depicted in Fig. 5A). (2) We compared these results with those from an ncDNA dataset similarly partitioned hierarchically into "minor" mtDNA clades (A–N) within "major" mtDNA clades ([A–D], [E–F], [G–H], [J–K], [N]). (3) Finally, to test population structure based on a model of divergence due to major rivers, we partitioned ncDNA into seven hypothesized populations isolated by six major rivers (Colorado, Red, Mississippi, Tombigbee, Choctawhatchee, and Apalachicola). We then grouped these populations in all possible ways, performing hierarchical AMOVAs on each partition scheme to determine which river(s) explain(s) the most variation. All AMOVAs were performed using Arlequin version 3.1.1 where significance of F_{ST} analogues was tested against 10,000 nonparametric permutations.

Partial Mantel tests were carried out using the ncDNA dataset for these same six rivers to test whether genetic distance is greater between samples on opposite sides of rivers than between equidistant samples from the same side (Smouse et al. 1986), a pattern expected if rivers restrict gene flow. For each river, great circle geographic distances and pairwise patristic genetic distances were calculated between genotypes originating from either side of the river. The partial correlation between genetic distance and a binary matrix (describing whether any two samples are separated by the river) was determined once autocorrelation due to geographic distance was accounted for. Tests were performed using the vegan package in R (Oksanen et al. 2009; R Development Core Team 2009) where significance was assessed using 1000 randomizations. We also performed Fisher's exact tests for mtDNA clade pairs putatively delineated by 10 major rivers (Colorado, Red, Atchafalaya, Mississippi, Amite, Pascagoula, Tombigbee, Choctawhatchee, Chipola, and Apalachicola/Chattahoochee) to test the significance of the association between rivers and clade membership.

DEMOGRAPHIC EXPANSION FROM REFUGIA

The presence of a signature of recent demographic expansion within the dataset was tested using a variety of methods. First, values of Tajima's D (T_D; Tajima 1989), Fu's F_S (Fu 1997), and Ramos-Onsins and Roza's R_2 (Ramos-Onsins and Rozas 2002) were calculated in Arlequin version 3.1.1 and DnaSP version 4.5 (Rozas et al. 2003) for all lineages and loci because significantly negative values can indicate recent growth. A statistical significance of these values was assessed with 10,000 coalescent simulations, where the estimated recombination rate (calculated in DnaSP) was assumed for diploid loci. The frequency distribution of pairwise genetic differences (or mismatches) within mtDNA clades was investigated using Arlequin version 3.1.1. We compared observed mismatch distributions against simulated distributions (expected to be unimodal under a model of sudden population growth; Rogers and Harpending 1992). Fit of the data to each model and confidence intervals around parameters were determined using the sum of squares deviations (SSD) between the observed and expected mismatch distributions as well as the raggedness index (Harpending 1994) calculated from 2000 bootstrap replicates.

We also reconstructed demographic histories for most mtDNA clades using the Bayesian skyline plot (BSP) as implemented in BEAST version 1.4.8 (Drummond and Rambaut 2007). The MCMC was run at least three times for at least 20 million generations (taking 10,000 samples per analysis) for each clade under a GTR + Γ + I model of evolution assuming a relaxed

uncorrelated lognormal molecular clock (Drummond et al. 2006). Effective sample size of parameters was assessed and plots were visualized using Tracer version 1.4.

We used LAMARC version 2.1.3 to produce posterior probability distributions of parameters within the exponential growth model $\theta_t = \theta_0 e^{-g t \mu}$ to assess population growth rate using the ncDNA dataset. Searches were run for each of the three major populations estimated by the program Structure. Several independent chains were run for 5–10 million generations, sampling every 100–140 steps, and convergence was assessed using Tracer version 1.4. Several initial runs were carried out to explore the parameter space, with final runs implementing a flat growth rate prior (linearly scaled) of $g = 1000$–5000 for population I and $g = 0$–4000 for populations II and III.

Finally, recent growth out of putative southern refugia would predict that southern populations exhibit higher levels of diversity than northern populations. To compare levels of diversity in these two regions while accounting for bias in sampling effort, we resampled 100 individuals (without replacement) from samples collected in the north and south (with 31° north latitude used as an arbitrary cut-off) separately for 500 iterations using R. We calculated mtDNA diversity indexes from each bootstrap using Arlequin version 3.1.1 and constructed distributions of values from which we obtained point estimates and 95% confidence intervals. We repeated this procedure for the eight nuclear loci (resampling from the reduced ncDNA dataset) and for individual mtDNA lineages (for which we had $\geq$15 samples north or south of 31°), except resampling only 20 and 12 individuals per iteration, respectively.

Results

DESCRIPTION OF GENETIC DATA

Excluding the outgroup, nuclear datasets contained between 20 and 70 variable sites of which 11 to 68 were parsimony-informative (Table S5). All six anonymous loci are assumed to be noncoding given they do not appear translatable into proteins or match any annotated gene regions in GenBank. Most haplotypes were reconstructed with 100% posterior probability, and no effect on results was observed when analyses were performed either including or excluding loci phased with <95% probability. GenBank accession numbers for all sequences are provided in Table S1.

PHYLOGENETIC AND NETWORK ANALYSIS

For the cyt*b* dataset, 314 unique haplotypes were recovered from 366 *S. lateralis* samples plus two outgroups. An absence of indels, nucleotide ambiguities, and stop codons indicates a mitochondrial origin for these sequences (Zhang and Hewitt 1996). Excluding outgroups, 389 sites are variable, of which 286 are parsimony-informative and likelihood ratio tests determined that GTR + Γ + I is the most likely model of evolution for the overall dataset. After burn-in trees were removed, codon-partitioned BI analysis produced a 50% consensus genealogy with a mean ln-likelihood of −10035.4 (standard deviation = 22.81). The ML phylogeny had a ln-likelihood of −9775.5 and a topology nearly identical to that recovered in the BI analysis. Only the BI tree is shown (Fig. 2), with BI posterior probabilities and ML bootstrap values indicated at the nodes. Phylogenetic analysis recovered 14 major monophyletic lineages that are largely concordant with geography (A–N; Figs. 1 and 3), with some additional phylogeographic substructuring evident (e.g., within A, G, J, and K).

The distribution of mtDNA lineages is shown in Figure 1, where the vast majority of diversity is observed along the Gulf Coast. The geographic extent of most clades is limited east–west, and clade boundaries often align with major rivers (Fig. 3). The six clades that extend north are longitudinally distributed such that panmixia is apparent for populations running north–south. There are 21 observed instances in which two or more lineages geographically overlap, and these are nearly always located near major rivers that otherwise delineate clade boundaries (Figs. 1 and 3). The most basal lineages are found in the southeastern portion of the range, with clades J–N all represented in the Florida panhandle, and with no members of these clades extending west of the Tombigbee River in western Alabama.

In contrast to mtDNA patterns, phylogeographic reconstruction for eight nuclear loci yields gene trees with few well-supported clades for most loci, significant discordance among trees (partition homogeneity test P-value = 0.01), and significant discordance with geography and cyt*b* lineages (data not shown). Haplotype networks for eight nuclear loci also reveal a large degree of haplotype sharing between geographically distant samples and genetically distant mtDNA clades, though some concordance with both is apparent (Fig. 4). Networks generally exhibit one or two dominant ancestral haplotypes, often represented throughout a majority of the geographical range, from which tip (and more geographically concordant) haplotypes derive. Each locus exhibits a distinct genealogical pattern though some agreement does exist among loci for particular relationships concordant with geographical regions (e.g., the Atlantic Coast or central Texas) or mtDNA clades (e.g., A, C, E, and F).

POPULATION DIVERSITY AND STRUCTURE

For cyt*b*, the mean within-population K2P divergence is 0.59% whereas mean between-population divergence is 6.8%, indicative of strong historical population fragmentation (Table S6). Assuming a 1% rate of evolution, mtDNA lineages diverged between 1.7 and 9.7 (1.6–8.0 uncorrected) million years ago (mya) or between 0.85 and 4.9 (0.8–4.0 uncorrected) mya, assuming a 2% rate. A

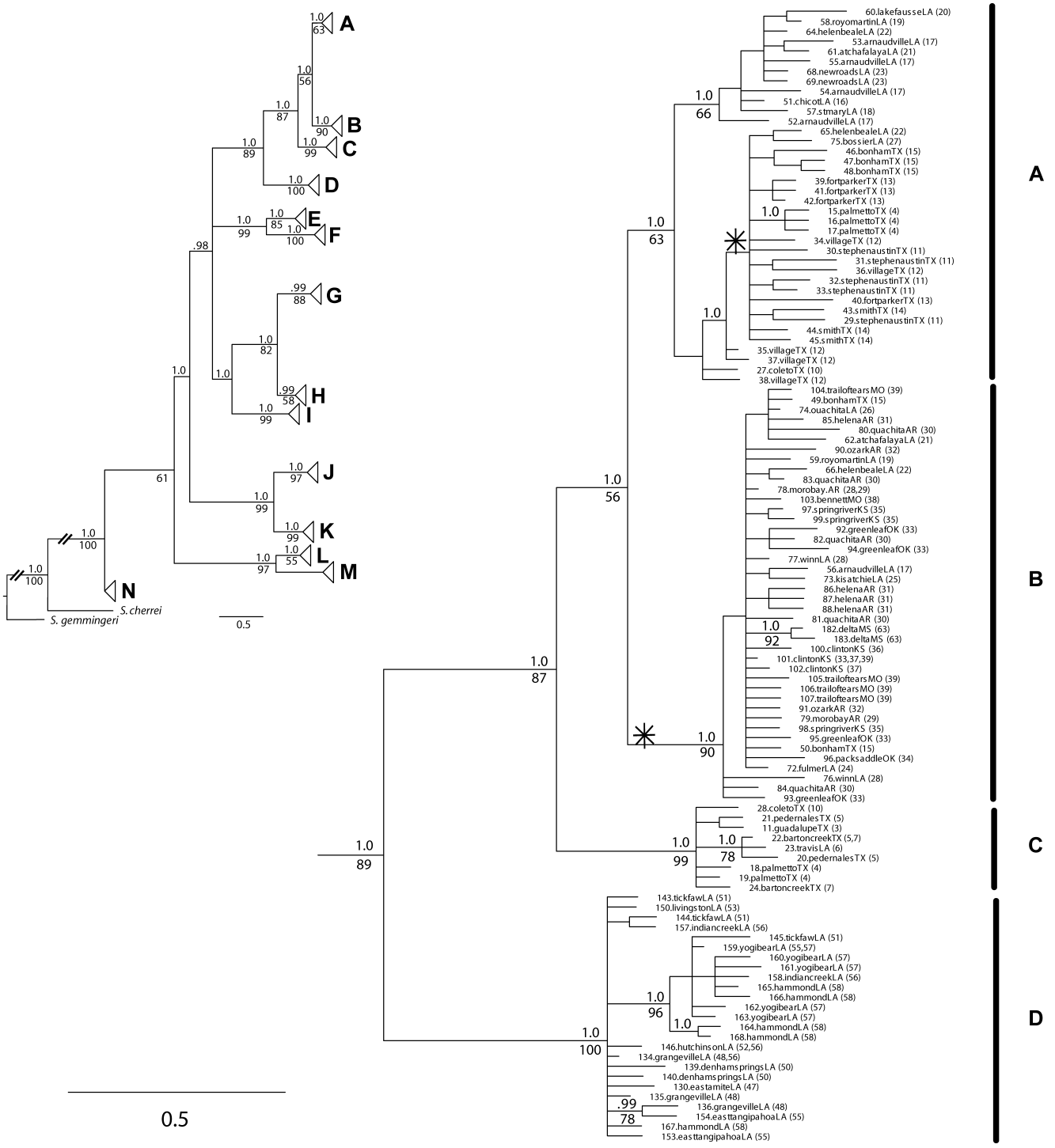

Figure 2. A 50% majority-rule consensus Bayesian phylogeny for 314 cytochrome *b* haplotypes plus two outgroups. Posterior probabilities ≥0.95 are indicated above and ≥50 ML bootstrap values indicated below select nodes. An outline phylogeny is included for reference and 14 clades are labeled A–N. Terminal haplotypes are labeled first with a haplotype number (correlating to numbering in Table S1) followed by a locality descriptor. Localities from which haplotypes were sampled follow parenthetically (correlating with locality numbers in Table S2). Double hatch marks indicate shortened branch lengths and nodes labeled with an asterisk indicate clades including haplotypes sampled above approximately 31° north latitude. Branch lengths are in substitutions per site.

Figure 2. Continued.

relative rates test suggests rate homogeneity across all lineages except clade I, which exhibited a significantly reduced rate ($P < 0.05$) relative to six other clades. Diversity indices for all loci are reported in Table S5.

For an initial set of Structure runs including the entire ncDNA dataset, a model assuming $K = 3$ populations best fits the data. Iteratively running Structure with incrementally increased values of K results in a peak in mean ln Pr(X | K) score at $K = 3$ (Pr [$K = 3$] = 1), after which scores plateau at larger values of K. Individuals were assigned, generally with high probability (>0.9), to one of three geographically distinct populations (I–III; Fig. 5A), though a longitudinally distributed zone of admixture is apparent between two of the populations in eastern Mississippi/western Alabama. For additional analyses of datasets partitioned according to their initial cluster assignment, populations II and III were best subdivided into $K = 2$ and $K = 3$ clusters, respectively, given peaks in ln probability scores at these values (posterior probability for both values = 1 when a uniform prior on K is used). Although yielding a low posterior probability relative to higher values of K, $K = 2$ populations was determined to best fit the data for population I because (1) larger values of K resulted only in the addition of purely admixed or empty clusters and (2) $K = 2$ is the modal value of ΔK, a statistic based on the rate of change in ln probability score between different K values (shown to strongly correlate with the true value of K; Evanno et al. 2005). The geographical distribution of individual assignments to these seven resulting populations is displayed in Figure 5B.

After the removal of recombining regions, 74.4% (between 50.8% and 100% for each locus) of the dataset was retained

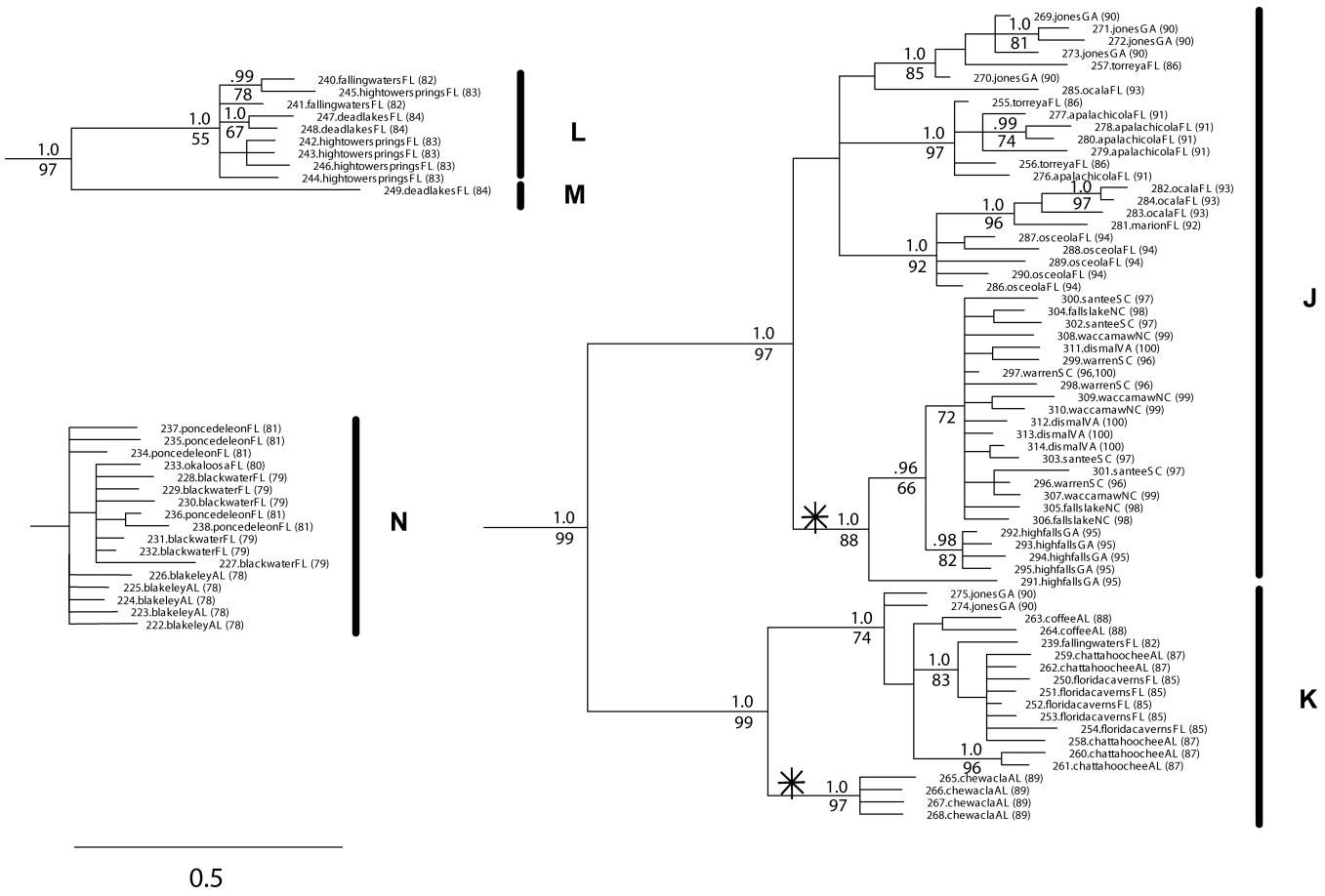

Figure 2. Continued.

and between 91.9% and 98.4% (average 95.7%) of gene copies were retained for each locus. Best estimates for θ and pairwise migration rates (estimated jointly) are given in Table 1. Assuming cyt*b*-calibrated mean nuclear mutation rates, N_e estimates are very large, ranging from 0.58 to 1.2 million, 3.8 to 7.6 million, and 2.7 to 5.3 million for populations I, II, and III, respectively. Although gene flow is apparent among all populations, the most migration occurs between populations II and III whereas population I is the most isolated.

ASSOCIATION OF DIVERSITY WITH RIVERINE BARRIERS

An AMOVA performed for ncDNA data partitioned according to populations assigned by the program Structure split similar amounts of the variation among "major" (26.15%, $P = 0.036$) and "minor" (23.24%, $P \leq 0.001$) population groupings. For the analysis partitioned in accordance to mtDNA lineage membership, "major" mtDNA phylogenetic structure explained as much variation as the "major" structuring inferred from the ncDNA itself (26.40%, $P \leq 0.001$), although "minor" mtDNA phylogenetic structure explained a smaller, though significant, proportion of the variation (12.20%, $P \leq 0.001$). The two river-based groupings that explained the most variation were groups made according to sample location relative to the Colorado and Red Rivers (among groups = 30.58%, $P = 0.049$ and among rivers = 14.60%, $P \leq 0.001$) and to the Colorado, Red, and Choctawhatchee Rivers (among groups = 33.88%, $P = 0.004$ and among rivers = 5.96%, $P \leq 0.001$). Partial Mantel tests indicate enhanced divergence associated with all rivers tested except the Mississippi, which did show a significant effect once samples from a contact zone in southeastern Louisiana were removed (Table 2). Fisher's exact tests reveal that mtDNA haploclade membership is better predicted by rivers than expected by chance (*P*-value ≤ 0.001 for all tests).

POPULATION EXPANSION FROM REFUGIA

Tajima's *D*, Fu's F_S, and Ramos-Onsins and Roza's R_2 were significantly negative for most mtDNA clades (Table S7) and all but one (P2–07) nuclear locus (Table S5). Mismatch distributions for all lineages were unimodal except for lineages A, G, and J. Given two evident subclades within both lineages A and J (Fig. 2), these were subdivided further into lineages A1, A2, and J1, J2 and reanalyzed, yielding unimodal distributions for all but clade J1. With one marginal exception (clade A), a model of population

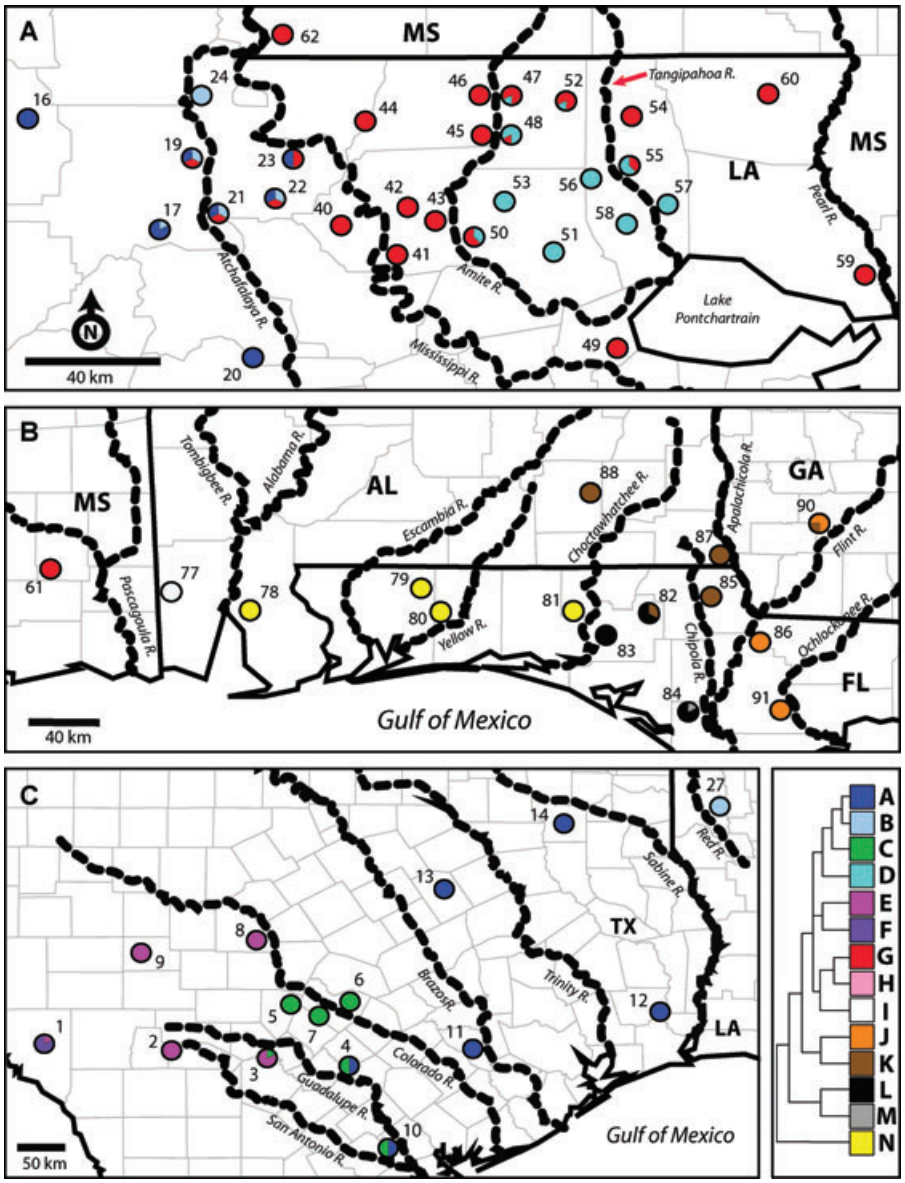

Figure 3. Geographical distribution of mtDNA clades in relation to major Gulf Coast rivers throughout (A) the Mississippi River Delta, (B) the Florida panhandle, and (C) west-central Texas. Numbers correspond to locality numbers in Table S2 and circle colors correspond to mtDNA clades, where localities harboring multiple clades are indicated by circles with mixed colors filled in proportion to clade representation.

growth could not be rejected for any lineage according to either sum of squares deviations or raggedness index tests (Table S7). Dates for expansion ranged from 100,000 to 1 million years ago, averaging 320,000 years (1% rate), or ranged from 50,000 to 500,000 years ago, averaging 160,000 years (2% rate; Table S7).

BSPs indicate that most populations have been slowly growing over the recent past. Lineages A, D, G, J, and K show surges in growth that began between 200–600 (1% rate) and 100–300 (2% rate) thousand years ago. Lineages B, C, E, H, N, and L exhibit a gradual growth trend over time not always statistically distinguishable from a model of demographic stationarity (Fig. S1). Estimates of the growth parameter *g* also suggest a history of extensive population growth for the entire species, with all confidence intervals excluding zero (Table 1B; Fig. S2). Although this parameter has been shown to be biased upward, our inclusion of multiple loci for the search should help to allay that bias (Kuhner and Smith 2007).

For the resampling analysis, nucleotide diversity (π) and haplotype diversity (Hd; here the 95% confidence intervals barely overlap) were significantly higher for populations south of 31° than populations north at cyt*b* (Table 3). Although confidence intervals overlap for most of the diversity estimates from other loci (Table 3) or individually treated mtDNA lineages (Table 4), point estimates and confidence intervals are almost always higher

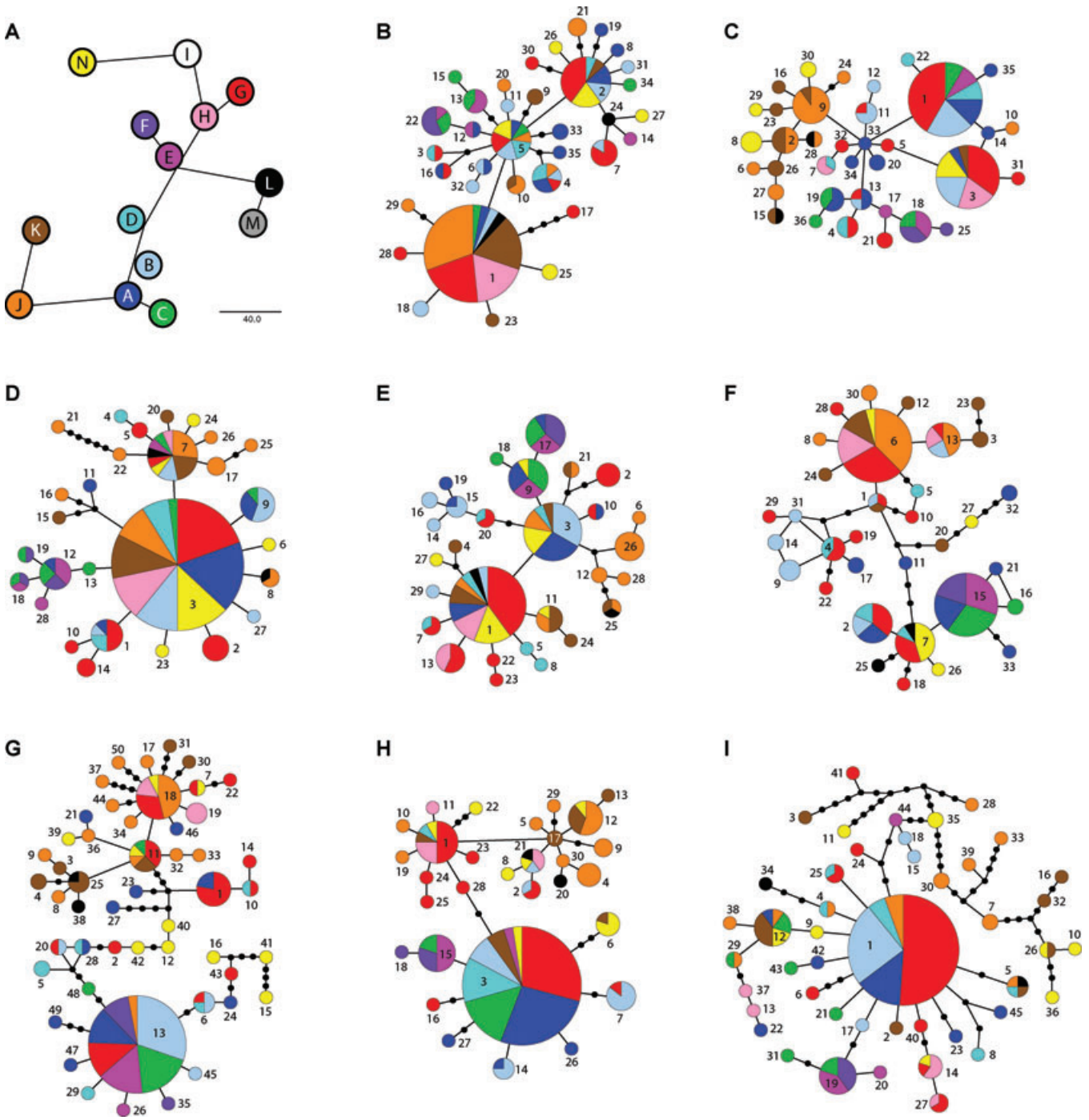

Figure 4. Gene histories for eight nuclear loci compared to the mtDNA phylogenetic pattern. (A) A minimum spanning tree generated in Arlequin showing the fourteen mtDNA clades discussed in the article. (B–I) Haplotype networks for eight nuclear loci (P1–18, P1–23, P1–58, P2–03, P2–07, P2–42, PRLR, and SELT, respectively; see Table S4) generated using the program TCS. Colored circles correspond to haplotypes where circle size is proportional to the number of samples per haplotype. Circle colors correspond to mtDNA clade colors as depicted in (A) (and in Figs. 1–3). Line segments between circles represent one mutational step and small black circles are inferred haplotypes not sampled. Numbers correspond to haplotype numbers given in Table S3. Network ambiguities (loops) were solved and eliminated in some instances either by inspection of haplotype networks generated using recombination-free blocks of data (see text) or by considering empirically validated predictions of coalescent theory (Crandall and Templeton 1993; Pfenninger and Posada 2002).

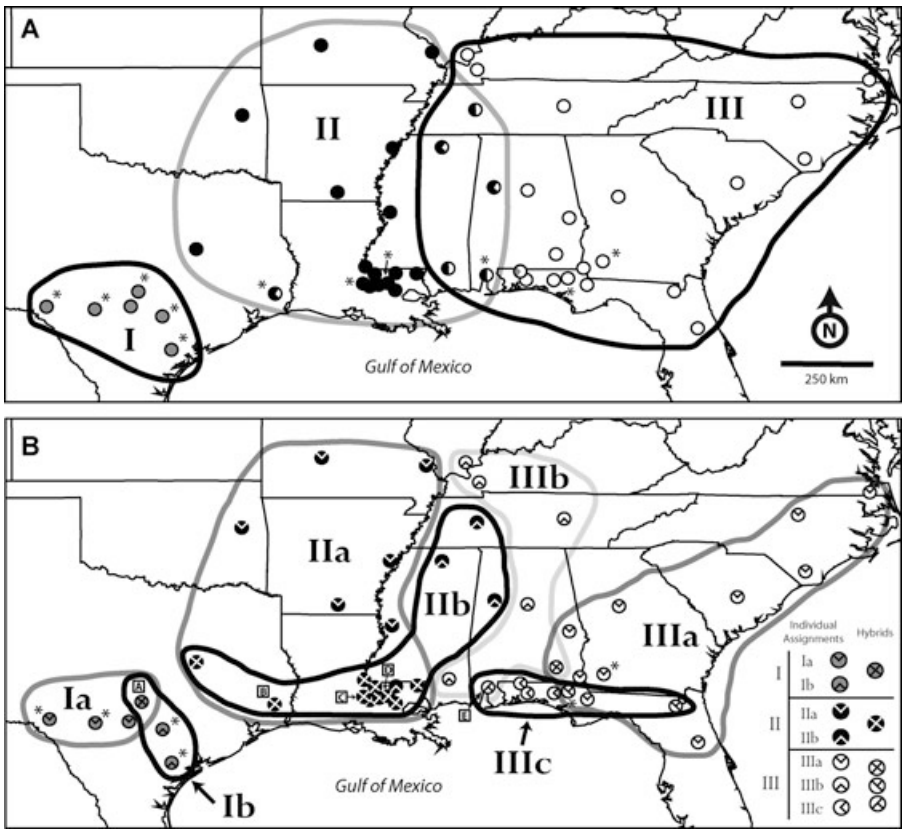

Figure 5. **Geographic distribution of population assignments inferred for eight nuclear loci using Bayesian clustering as implemented in the program Structure. (A) Results from analysis of the entire dataset and (B) results from analysis of datasets partitioned in accordance with populations I–III depicted in (A). Circles correspond to sampling localities, circle fill corresponds to sample assignment to one of three populations (I–III), and line design within circles corresponds to sample assignment to one of seven subpopulations (Ia–Ib, IIa–IIb, or IIIa–IIIc). Hybrid samples (any sample assigned to two populations with ≥20% probability) in (A) are depicted with mixed fill roughly proportioned to the proportion of assignment probabilities. Hybrid samples in (B) are depicted with combined population line designs. Asterisks indicate localities from which two or more individuals were sequenced that had the sample population assignment probability profile. Small squared letters indicate localities from which two or more individuals were sequenced that had different assignment profiles, one assigned as indicated on the map (hybrids in all cases) and the others assigned as follows: A = Ia, B = IIb, C = IIa, IIb, IIb, IIb, D = IIb, and E = IIIc.**

in southern over northern groups and estimates of τ (relative time since population growth) are also generally highest in southern populations in support of more recent growth in the north (Table 4).

Discussion

The importance of Pleistocene climate change on diversification of the biota inhabiting unglaciated eastern North America is well documented (Auffenberg and Milstead 1965; Hewitt 2000; Soltis et al. 2006). The power of major rivers in the region beyond the very largest (e.g., Mississippi, Apalachicola, and Tombigbee Rivers) to shape the evolution of these same taxa is less well-established, possibly in part due to the confounding effects of climate change. By driving range shifts and population extinction, periodical climatic fluctuations can reduce and eliminate genetic diversity that has (or would have) developed due to large rivers encountered under more stable conditions, causing us to underestimate the isolating force of rivers. On the other hand, this same climate change may be integral to the barrier effect of rivers, and many rivers may lose their ability to isolate populations once species are released from Pleistocene refugia trapping them near the coast where rivers are most impenetrable. The ultimate effect of the Pleistocene on a particular taxon is thus dependent on a variety of interacting ecological, genetic, or geographical factors.

Here, we report a case of iterative population fragmentation for a scincid lizard that is best explained by a combination of Plio-Pleistocene refugia and riverine barriers. An east–west string of 14 monophyletic mtDNA lineages has been recovered along the Gulf Coast, largely concordant with major rivers, that have all apparently survived the Pleistocene glaciation cycles, indicating a more continuous supply of refugia along the Gulf Coast than has been previously appreciated (Jackson et al. 2000;

Table 1. Parameter estimates for three *S. lateralis* populations from LAMARC's Metropolis–MCMC searches applied to eight nuclear loci. (A) Most probable estimates (with 95% confidence intervals) of pairwise migration rates in units of $4N_e m\mu$. (B) Most probable estimates (with 95% confidence intervals) of θ and growth rate g for the three populations.

(A)		TO		
		Pop I	Pop II	Pop III
FROM	Pop I	*	0.6350 (0.005647–2.466)	0.002597 (0.0004315–0.8367)
	Pop II	0.5021 (0.0001059–1.988)	*	1.461 (0.06898–4.075)
	Pop III	0.002159 (0.0009751–0.5773)	1.745 (0.07860–5.067)	*

(B)	Pop I	Pop II	Pop III
θ	0.008356 (0.005202–0.014867)	0.05451 (0.03845–0.07531)	0.03805 (0.02632–0.05512)
G	2204.7 (1188.93–4405.50)	2127.1 (1562.74–2982.20)	2493.9 (1749.9–3592.4)

Loehle 2007; Gonzales et al. 2008). The observed phylogeographic pattern placing nearly all genetic diversity observable today along a narrow strip near the gulf suggests that much of the species diversity originated from diversification along the southern coast and that northern populations result from either recent or recurrent expansion from this southern strip of diversity. Diversity of the species appears to have originated in the Florida panhandle where three of the most basal clades reside, and later expanded west through the Mississippi River Valley and into Texas perhaps at a time when warmer, moister conditions allowed circum-navigation of major rivers.

The multilocus ncDNA pattern is broadly concordant with the mtDNA pattern suggesting that allele-sharing exhibited among ncDNA populations is primarily due to a difference in evolutionary tempo (slower sorting) rather than trajectory (differential gene flow) between the two genomes. The geographical boundaries of ncDNA populations IIa, IIIa, IIIb, and IIIc inferred by a cluster analysis are broadly concordant with those of mtDNA clades B, J-K, H, and N, respectively. Also, structuring multilocus variation using AMOVA in accordance with Structure clusters or deeper-level mtDNA lineages explains similar proportions of ncDNA variation, whereas structuring ncDNA variation according to more shallow mtDNA divergences explains much less of the variation, a pattern expected if incomplete sorting causes discordance (given that deeper divergences should be more complete than recent divergences). Assessment of ncDNA-mtDNA concordance for geographically restricted mtDNA lineages (e.g., D, F, I, K, L, and M) awaits additional multilocus sampling beyond the 0 to 3 for each lineage used here.

Table 2. Pearson's correlation coefficients (*r*) and *P*-values for partial Mantel tests carried out for samples on the opposite sides of six rivers. These tests estimate the correlation between genetic distance and river location, given the geographic distance between samples.

River	*r*	*P*-value
Apalachicola	0.1546	0.012
Choctawhatchee	0.4109	0.001
Colorado	0.4109	0.001
Mississippi	−0.00877	0.584
Mississippi[1]	0.8314	0.001
Red	0.3366	0.011
Tombigbee	0.4205	0.001

[1]A test repeated for the Mississippi River with samples in southeastern Louisiana removed.

EVIDENCE FOR RIVERINE BARRIERS

The riverine barrier hypothesis predicts that boundaries of diversity will correlate with the location of major rivers (Sick 1967). For mtDNA, this appears to be the case, where Fisher's exact tests indicate that *S. lateralis* clades are significantly concordant with 10 major rivers along the Gulf Coast (see Fig. 3). Of the major rivers flowing into the Gulf of Mexico within the range of *S. lateralis*, the five largest (as measured by mean discharge; Benke and Cushing 2005) harbor divergent clades on opposite sides (Mississippi, Atchafalaya, Tombigbee, Apalachicola, and Pascagoula). Other rivers that appear to delineate lineages are also among the major rivers near the coast (Choctawhatchee, Red, and Colorado) or are tributaries to major rivers that may have carried a much larger portion of drainage flow in the past (Amite and Chipola). AMOVA and partial Mantel tests show that multilocus diversity is also significantly associated with major rivers (Table 2). Defining population structure by sample location in relation to the Colorado, Red, and Choctawhatchee Rivers explains an amount of the genetic variation (33.9%), that is comparable to that explained by the three major Structure-inferred populations (26.2%) or by five major mtDNA clades (23.2%). Finally, the geographic distribution of lineage overlap observed is also suggestive of a role for riverine barriers, with zones of contact almost always occurring along rivers. Where we sampled close to rivers, some populations near the Guadalupe, Colorado, Red, Atchafalaya, Mississippi,

Table 3. Point estimates and 95% confidence intervals (CI) from distributions of diversity indices calculated across nine loci for 500 iterations of haplotype resamplings taken separately from north and south of 31° north latitude.

Region	Locus	Hd	Hd CI	*k*	k CI	π	π CI
North	Cytb	0.9961	0.9941–0.9977	50.8481	49.0374–52.2826	0.0445	0.04290–0.04574
South	Cytb	0.9987	0.9976–0.9996	60.2910	57.8699–62.3097	0.0526	0.05045–0.05432
North	P1–18	0.9057	0.8263–0.9578	2.1384	1.3315–2.9210	0.0046	0.00285–0.00621
South	P1–18	0.9189	0.8315–0.9736	2.7168	2.1368–3.2736	0.0058	0.00454–0.00698
North	P1–23	0.9086	0.8368–0.9578	2.1931	0.8684–3.1000	0.0042	0.00165–0.00593
South	P1–23	0.9627	0.9157–0.9894	3.7049	2.9263–4.4368	0.0070	0.00550–0.00861
North	P1–58	0.8614	0.7526–0.9421	1.0053	0.4000–1.4368	0.0023	0.00091–0.00330
South	P1–58	0.9155	0.8315–0.9736	1.6706	0.8315–2.9894	0.0038	0.00193–0.00681
North	P2–03	0.9241	0.8789–0.9578	2.3306	1.5210–3.1210	0.0045	0.00293–0.00588
South	P2–03	0.9335	0.8684–0.9736	2.4083	1.5684–3.2526	0.0046	0.00302–0.00612
North	P2–07	0.9646	0.9368–0.9842	3.1543	2.1842–3.9105	0.0055	0.00380–0.00680
South	P2–07	0.9726	0.9263–0.9947	4.2589	3.2526–5.0421	0.0075	0.00566–0.00884
North	P2–42	0.8872	0.8157–0.9368	6.4778	5.2000–7.4368	0.0105	0.00840–0.01201
South	P2–42	0.9357	0.8473–0.9947	7.1325	3.7789–8.7526	0.0122	0.00638–0.01553
North	PRLR	0.9360	0.8947–0.9631	2.8965	2.4789–3.3105	0.0052	0.00445–0.00594
South	PRLR	0.8215	0.6473–0.9263	2.0965	1.4894–2.6052	0.0038	0.00267–0.00466
North	SELT	0.8263	0.7000–0.9263	4.1283	2.8473–5.2578	0.0049	0.00340–0.00628
South	SELT	0.9217	0.8368–0.9789	3.8261	1.9105–5.2210	0.0049	0.00321–0.00686

Hd, haplotype diversity; k, mean no. of pairwise differences; π, nucleotide diversity.

and Amite Rivers were found to consist of mixed ancestry indicating some degree of permeability to dispersers (Figs. 1 and 3).

A riverine basis for divergence also predicts that larger, longer rivers will delineate populations further from the mouth than smaller, shorter rivers (Ayres and Clutton-Brock 1992). Of the 10 rivers that correspond with mtDNA lineage boundaries near the coast, the four rivers that continue to delineate clade boundaries in the north (above approximately 31° north latitude; Red, Mississippi, Tombigbee, and Apalachicola/Chattahoochee Rivers) are within the top five largest of these (as measured by mean discharge; Benke and Cushing 2005). However, contrary to expectations of the riverine barrier hypothesis, relatively smaller rivers such as the Colorado and Red Rivers explain more multilocus structure than the Mississippi and Apalachicola Rivers (Table 2). One reason for this is likely the periodic channel-switching of the lower Mississippi River since the Pleistocene (Fisk 1944; Kolb and Van Lopik 1966) leading to geographic

Table 4. Point estimates and 95% confidence intervals (CI) from distributions of diversity and demographic indices calculated from 500 iterations of resampling across 10 lineages located either north or south of 31° north latitude.

	Clade	Hd	Hd-CI	*k*	k CI	π	π CI	τ	τ CI
North	B	0.9812	0.9394–1	5.6869	3.7727–8.1212	0.00496	0.00331–0.00709	5.0615	3.3848–6.7031
	G	1	NA	5.5140	4.37879–6.5152	0.00481	0.00382–0.00569	5.2446	3.8594–6.4609
	H	0.9763	0.9394–1	5.7306	4.4849–6.8939	0.00500	0.00391–0.00602	4.8649	2.5527–7.9453
	J	0.9898	0.9546–1	5.7608	4.0909–7.5303	0.00503	0.00357–0.00657	5.2703	3.3418–6.9688
South	A	1	NA	8.6584	7.40909–9.7272	0.00755	0.00646–0.00848	7.0171	4.6699–10.3145
	D	0.9866	0.9697–1	6.4643	5.0909–7.5455	0.00564	0.00444–0.00658	5.0616	2.6582–8.0234
	G	0.9903	0.9545–1	8.0166	6.4091–9.6212	0.00699	0.00559–0.00839	9.8068	3.3242–12.5039
	J	0.9910	0.9697–1	15.9885	13.9091–17.8636	0.01399	0.01217–0.01563	20.0482	17.8555–22.2891
	K	1	NA	7.3347	6.1818–8.2273	0.00640	0.00539–0.00718	7.9327	3.2070–9.6758
	N	1	NA	6.6068	5.6515–7.4848	0.00576	0.00493–0.00653	6.6801	5.4883–7.6387

Hd, haplotype diversity; k, mean no. of pairwise differences; π, nucleotide diversity; τ, relative time in generations since population growth.

overlap of two major mtDNA clades (A-B and G between the Mississippi and Atchafalaya Rivers) and two ncDNA populations (IIa and IIb spanning the Gulf Coast from eastern Louisiana to east Texas) otherwise separated by the Mississippi River Valley (Figs 3 and 5B). Repeating a partial Mantel test with southern Louisiana samples removed produces a correlation coefficient at least twice that of any other river (Table 2). A past channel-switching event of the Apalachicola River (Donoghue 1989) may likewise have left mtDNA clade K separated from its sister clade (J) west of the river (where populations could have survived cyclical flooding in the Grand Ridge uplands; Fig. 3), thus resulting in the lower observed multilocus divergence directly east and west of that river. Also, given that lineage sorting is expected to progress most quickly in peripheral populations (Gavrilets et al. 2000), the enhanced divergence for the Colorado and Red Rivers (which delineate peripheral, lower density, populations) may result more from this "periphery effect" than from reduced migration across these rivers.

A potential exception to the riverine basis of vicariance is in Texas, where rivers are narrower and the river-lineage correlation is more dubious, especially in regard to the origin of clades E and F (Fig. 3C). In this case, given *S. lateralis*'s patchier distribution in central Texas due to a heightened reliance on riverine environments, expanses of dry habitat may impose a greater isolating force than rivers in this region. Also, without additional sampling, a role of local adaptation (to the relatively dryer climates of central Texas) in contributing to divergence from eastern populations cannot be ruled out (Endler 1982).

EVIDENCE FOR REFUGIA

The refugia hypothesis predicts both the highest genetic diversity within and a signature of recent growth out of populations residing in putative refugia (Capparella 1991; Starkey et al. 2003; Fuerst and Austin 2004). In support of this, nearly all genetic diversity is found near the Gulf Coast, with every major lineage but one being represented south of approximately 31° north latitude (and given further sampling, the remaining lineage, H, may also conform to this pattern), and only six being observed north of 31° (Fig. 1). Of the six clades that extend north, usually only a subset of the diversity of these lineages is observed north of 31° (Fig. 2) and no substantial phylogenetic structure exists north of 31° north latitude that is not represented south of that mark (excepting the aforementioned clade H). Given uneven sampling within lineages and regions as well as some lineages that span north and south, it is difficult to compare diversity and demographic indexes between different latitudes (Table S7). However, iterative resampling from these two regions also supports the idea that genetic diversity is highest near the coast and that recent demographic growth commenced most recently in the north, expected if growth progressed from lower to higher latitudes (Tables 3 and 4).

Multiple methods and gene regions support the hypothesis of recent growth for most *S. lateralis* populations (Table 1B and S7; Figs. S1 and S2). A 1–2% evolutionary rate places the genesis of these expansions within the Pleistocene (anywhere from approximately 50,000 to 1 mya). Although these dates are derived from the problematic assumption of a molecular clock, these combined dates do reject the simple scenario positing genetic bottlenecks in southern refugia during the last glacial cycle followed by multiple northward range expansions after the last glacial maximum (LGM) approximately 12,000 ybp. Such a scenario would require evolution rates more than an order of magnitude greater than has been reported for lizards to explain date estimates obtained from a variety of methods and loci. This does not rule out a more coastal distribution for the species during glacial periods followed by a recent post-LGM expansion of some lineages further north. Some anecdotal evidence suggests that *S. lateralis* populations may even now be expanding their ranges further northward (Moore 1896; Myers 1959). However, this does suggest that expanding populations are sequestering genetic variation that has likely survived the most recent Wisconsin glacial cycle (approximately 12–110 ka), and possibly even the Illinoian (approximately 130–200 ka), Kansan (approximately 300–455 ka), and Nebraskan (approximately 620–680 ka) cycles as well. The species' capacity for retaining high levels of variation in times of reduced range size is not necessarily surprising given very large estimates of effective population size (between approximately 0.5 and 7.5 million; Table 1B).

RIVERINE BARRIERS STRENGTHENED BY REFUGIA

The phylogeographic pattern observed for *S. lateralis* is unusual and suggests that two different evolutionary histories have taken place in the northern and southern portions of the species range. Near the coast, south of approximately 31° north latitude, rivers appear to have served as effective isolating barriers (and refugial boundaries) helping to generate a latitudinal strip of at least 13 distinct mtDNA lineages. In contrast, the phylogeographic pattern in the north is more similar to that seen in other codistributed taxa (e.g., Soltis et al. 2006; Burbrink et al. 2008) with major genetic breaks at the Mississippi and Apalachicola/Chattahoochee Rivers, along with some substructuring concordant with Tombigbee and Red Rivers. The reduced structure in northern populations implies that many rivers that are isolating near the coast are circumnavigable further north, begging the question of how some rivers were able to adequately isolate populations such that long-term divergence was possible. This pattern suggests a temporally two-tiered evolutionary history for the species, with initial population vicariance and divergence taking place when populations were geographically limited to the southern portions of their current range where rivers are widest and most impenetrable to migration, followed later by expansion of populations to the north where

a few demographically dominant mtDNA clades now remain. The apparent consistency in the latitudinal extent of all lineages endemic to the coast (estimated around 31° north latitude) speaks to a single unifying cause that limited northward expansion beyond the coast equally for all lineages.

The river–refugia hypothesis posits that rivers alone are insufficiently impermeable to migration to genetically divide populations and thus predicts that riverine-based divergence must have commenced once populations were forced into refugia. Given the reliance of *S. lateralis* on warm-temperate mesic habitats (Milstead 1960; Ashton and Ashton 1985), cooler, more arid climates in the Plio-Pleistocene are a likely mechanism by which populations may have been trapped into coastal refugia. Assuming a cyt*b* evolution rate of 2% places most lineage divergences in the mid- to late Pliocene, a time when North America was beginning to become generally cooler and more xeric (Hibbard 1960; Frakes 1979). Invoking a slower evolutionary rate of 1% places most of these divergences in the late Miocene-early Pliocene, a period also thought to coincide with marked global cooling and reductions in sea level (Adams et al. 1977; Frakes 1979; Vail and Mitchum 1979).

Though most southeastern rivers were probably less capable of initiating diversification of populations able to freely disperse far north of the coastal plain during warmer, humid periods, interglacial cycles likely also contributed to current patterns of fragmentation. For instance, elevated riverine discharge, widened basins, and rising sea levels (leading to marine embayment of drainages and headwaters forced northward) during periods of deglaciation (Donoghue 1989; Dowsett and Cronin 1990) may have helped maintain riverine barrier effects in the face of simultaneous northward expansion of populations (Marroig and Cerqueira 1997; Pauly et al. 2007). Isolated islands of upland habitat surrounded by lowland flooding during high sea stands may have also played a diversifying role for some lineages (Neill 1957; Nores 1999; Means and Krysko 2001). However, the continued isolation of these lineages during times of aridification would likely have been aided by climate-induced restriction of populations to regions south of riverine headwaters.

Many codistributed species were likewise forced south due to past climate change (Auffenberg and Milstead 1965), yet these lineages lack the unique pattern of mtDNA diversification seen in *S. lateralis* (Soltis et al. 2006). The exceptional fragmentation observed in this group along the Gulf Coast may have originated from either higher-than average diversification rates prior to (and continuing throughout) the Pleistocene or lower-than average extinction rates during the Pleistocene relative to codistributed taxa. Enhanced divergence due to rivers could be explained by a more limited ability of *S. lateralis* to cross large rivers or a greater historical sensitivity to temperate climates (leading to a more southern distribution in the past and thus stronger riverine barrier effect) than exhibited by other species. Reduced extirpation of genetically distinct populations for *S. lateralis* would suggest that rivers were also important drivers of divergence in other taxa, but that only a limited number of refugia (such as the Mississippi River Valley, Peninsular Florida and Mexico; Blair 1958; Jackson et al. 2000) were able to sustain viable populations for most of them throughout the Pleistocene. Thus, the current lineage diversity for many species observed today would represent only a subset of the diversity in existence pre-Pleistocene. Exceptionally large population sizes observed for *S. lateralis,* along with the species' continuous, abundant distribution throughout the Gulf Coast (due to its high tolerance to a wide variety of mesic habitats) is consistent with an unusual propensity to survive Pleistocene climate change in a large number of coastal refugia.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Bayesian skyline plots constructed in the program BEAST depicting recent demography reconstructed against time for some mtDNA lineages.

Figure S2. A plot of population growth over time constructed for three populations using growth rate estimates (*g*) obtained from LAMARC's Bayesian search algorithm applied to data from eight nuclear loci.

Table S1. List of all specimens used in this study.

Table S2. List of localities sampled for this study.

Table S3. List of all specimens sequenced for eight nuclear loci and their haplotypes.

Table S4. List of primers used in this study.

Table S5. Diversity indices across loci used in this study calculated from 367 (cyt*b*) and 62 (nuclear loci) individuals (outgroups were excluded) using Arlequin and DnaSP.

Table S6. Average pairwise distances within and among 14 mtDNA lineages.

Table S7. Diversity indices and mismatch analysis results carried out for mtDNA clades using Arlequin and DnaSP.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).