

Lineage diversification in a widespread species: roles for niche divergence and conservatism in the common kingsnake, *Lampropeltis getula*

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Abstract

Niche conservatism and niche divergence are both important ecological mechanisms associated with promoting allopatric speciation across geographical barriers. However, the potential for variable responses in widely distributed organisms has not been fully investigated. For allopatric sister lineages, three patterns for the interaction of ecological niche preference and geographical barriers are possible: (i) niche conservatism at a physical barrier; (ii) niche divergence at a physical barrier; and (iii) niche divergence in the absence of a physical barrier. We test for the presence of these patterns in a transcontinentally distributed snake species, the common kingsnake (*Lampropeltis getula*), to determine the relative frequency of niche conservatism or divergence in a single species complex inhabiting multiple distinct ecoregions. We infer the phylogeographic structure of the kingsnake using a range-wide data set sampled for the mitochondrial gene cytochrome *b*. We use coalescent simulation methods to test for the presence of structured lineage formation vs. fragmentation of a widespread ancestor. Finally, we use statistical techniques for creating and evaluating ecological niche models to test for conservatism of ecological niche preferences. Significant geographical structure is present in the kingsnake, for which coalescent tests indicate structured population division. Surprisingly, we find evidence for all three patterns of conservatism and divergence. This suggests that ecological niche preferences may be labile on recent phylogenetic timescales, and that lineage formation in widespread species can result from an interaction between inertial tendencies of niche conservatism and natural selection on populations in ecologically divergent habitats.

Keywords: biogeography, genetic divergence, niche conservatism, niche modelling, speciation

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Introduction

Speciation across geographical barriers can be influenced by both niche conservatism (i.e. Peterson *et al.* 1999; Kozak & Wiens 2006) and niche divergence in ecologically distinct habitats (i.e. Graham *et al.* 2004; Raxworthy *et al.* 2007; Rissler & Apodaca 2007). The idea that ancestral niches may be conserved across evolutionary time (phylogenetic niche conservatism; Ricklefs

& Latham 1992; Wiens 2004) has recently gained a great deal of currency in the literature on speciation and the study of broad scale patterns of lineage formation (Peterson *et al.* 1999; Wiens & Graham 2005; Hawkins *et al.* 2006). Niche conservatism promotes allopatric divergence in fragmented habitats by limiting adaptation to new environments when populations maintain an ancestral niche. Alternatively, niche divergence may lead to lineage formation when populations adapt to new environments (Wiens 2004; Wiens & Graham 2005). The mechanism by which this happens is well defined, in terms of the fragmentation of a continuous

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habitat, which promotes geographical isolation, or dispersal into a new habitat, after which natural selection subsequently promotes ecological differentiation (Futuyma 1998; Coyne & Orr 2004). However, the relative importance of these processes for promoting lineage formation in single widespread species complexes has yet to be fully investigated. Whether both adaptive divergence and ancestral conservatism of ecological niche can influence lineage formation in widespread taxa crossing multiple distinct ecoregions and putative geographical barriers remains unknown.

At least three scenarios for allopatric speciation are possible when considering the roles of geographic barriers and ecological niche. The first is the presence of a geographical barrier dividing ecologically distinct populations, which indicates niche divergence across the barrier (e.g. Graham *et al.* 2004; Raxworthy *et al.* 2007). The second scenario involves a barrier that divides ecologically similar populations, and indicates phylogenetic niche conservatism (e.g. Kozak & Wiens 2006). The final scenario is the presence of ecologically divergent populations on a continuous landscape without a physical barrier separating them, indicating population divergence promoted or at least reinforced by niche divergence (e.g. Gee 2004). While these patterns have been demonstrated on local geographic scales in various taxa, it is unknown how broadly distributed organisms, which inhabit a diversity of niches across their range, respond to physical barriers and environmental variation, and whether one or a combination of the above scenarios is the dominant mode of allopatric divergence within in wide-ranging taxa.

Ectotherms such as reptiles are well suited for assessing the impact of environment and geography on lineage formation because of their low vagility and strong responses to environmental factors (e.g. Howes *et al.* 2006; Burbrink *et al.* 2008; Fontanella *et al.* 2008). The common kingsnake (*Lampropeltis getula*) is transcontinentally distributed in North America (NA; Conant & Collins 1998; Krysko 2001) and also appears ideal for investigating the roles of geographical barriers and ecological niche on the formation of lineages because of its ancient occupation of this region. Fossils have been found in the central part of the U.S. dating to the late-Miocene (Holman 2000) and by the Pliocene, the kingsnake had attained a distribution similar to the one inhabited today (Holman 2000; Parmley & Walker 2003).

To examine phylogeographic structure in the North American kingsnake, we assembled a range-wide molecular data set of the mitochondrial gene cytochrome *b* (*cyt-b*). While many issues regarding the use of single mitochondrial gene estimates of phylogeny have been raised (see Edwards & Beerli 2000), the use

of mtDNA for estimating phylogeographic structure still has advantages (see Brito & Edwards 2008; Zink & Barrowclough 2008), particularly for tracking recent population divergence and associated ecological influences. While issues such as stochastic gene tree/species tree discordance may influence phylogenetic estimates, this does not directly affect the inference of local genetic structure using mitochondrial loci.

First, we use phylogenetic reconstructions and divergence time estimates to characterize the geographical population structure in the kingsnake relative to the presence of putative physical barriers in the range of the organism. Second, given the possible uncertainty in tree structure because of variance in the coalescent and the ancient age by which the current range had been attained (Pliocene), we test a scenario of structured divergence across North America derived from our maximum-likelihood tree of the real data vs. a null model of an unstructured widespread ancestor. Rejecting the null model will permit us to determine which clades can provide robust comparisons of ecological niche characteristics.

Similar to Kozak & Wiens (2006, 2007), we use statistical methods developed for assessing ecological niche models (ENMs) to infer processes of niche conservatism and niche divergence in the formation of lineages, where empirical results in the kingsnake may exhibit any or all of the following three patterns: (i) niche conservatism at a physical barrier; (ii) niche divergence at a physical barrier; and (iii) niche divergence between ecoregions that lack physical barriers. Using predicted habitat suitability as a proxy for ecological niche, a lack of statistically significant differences between ENMs for lineages divided by geographical barriers would represent the signature of niche conservatism. Significant differences in niche at a geographical barrier would represent niche evolution or divergence in allopatry. Significant niche differences in the absence of a physical barrier would indicate that lineage formation was promoted, or at least maintained, by niche divergence in ecologically heterogeneous environments.

Methods and materials

Sequence acquisition

We obtained 201 tissue samples of *Lampropeltis getula* taken throughout their known range and downloaded 60 gene sequences from a previous systematic study of *L. getula* (Krysko & Judd 2006) from GenBank (File S1) for a total of 261 samples (Fig. 1). For the tissue samples, we used standard methods of proteinase K digestion in lysis buffer followed by several rounds of phenol/CHCl₃ extraction (Sambrook & Russell 2001) or

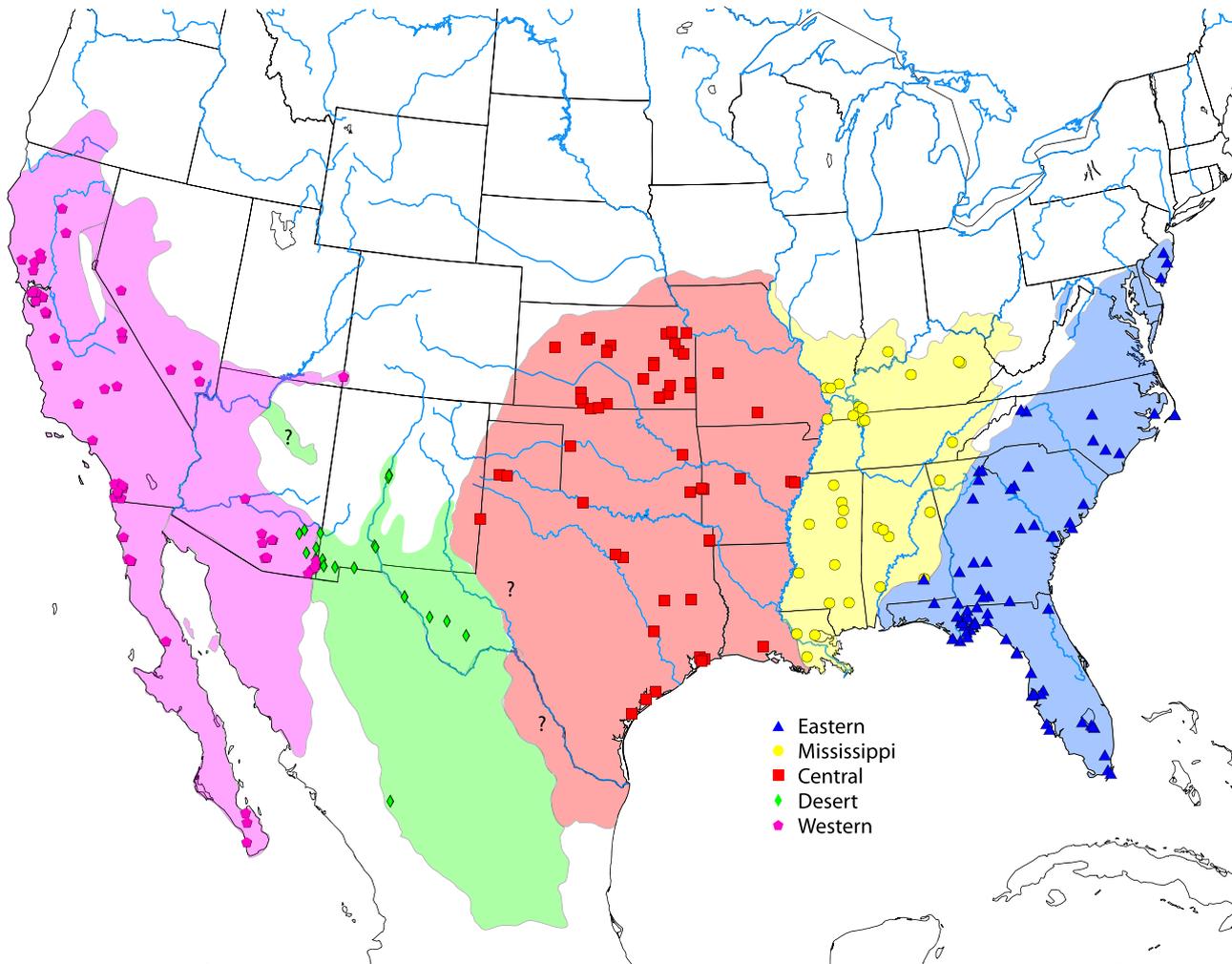


Fig. 1 Map of North America showing the sample localities, estimated range and identification legend for the major geographical lineages identified in this study. Range limits adapted from Conant & Collins (1998), Stebbins (2003) and the results of the distribution modelling.

Qiagen DNEasy kits (tissue protocol) to obtain total genomic DNA from samples of shed skin, liver or muscle tissue or whole blood. The complete mitochondrial gene *cyt-b* was amplified using GoTaq Green Master Mix (Promega Corp.) according to the manufacturer's specifications, with a 90-s extension time. The polymerase chain reaction (PCR) products were cleaned using 1 μ L of ExoSap-IT (USB Corp.) per 10 μ L of PCR product. The sequencing reaction consisted of 3 μ L Beckman-Coulter DTCS, 2 μ L primer (5 μ M), 3 μ L template and 2 μ L deionized water. Primers for the PCR and cycle sequencing reactions were as follows; *cyt-b* amplification: H14910 and THRSN2 (Burbrink *et al.* 2000), sequencing: LampSeq1F (5'-GTA ATT ACA AAC CTA CTA ACA GC-3') and LgetSeqRev2 (5'-TTT GTT CCT ART GGG TTR CTA GAG-3'). For some particularly old or degraded templates, *cyt-b* was amplified and sequenced in two fragments, using H14910 + LgetSeq-

Rev2 and LampSeq1F + THRSN2. Nucleotide sequences were examined and aligned by eye using the program SEQUENCHER 4.5 (Genecodes 2000). No sequences contained any gaps or any stop codons that would have suggested a pseudogene.

Phylogenetic inference

To test for the presence of population divergence and assess the potential impact of geographical features on lineage formation, we inferred the phylogeographic structure of *L. getula* using Bayesian inference (BI) and Maximum-Likelihood (ML) methods. For Bayesian phylogenetic inference, we used the program MRBAYES version 3.1.2 (Huelsenbeck & Ronquist 2001) to infer trees and nodal support. To assess model complexity, we tested a codon position partitioned General Time Reversible model with gamma distributed rate heterogeneity and a

proportion of invariant sites (3GTR + Γ + I) against a codon position partitioned HKY85 + Γ + I model using Posterior Bayes Factors (Kass & Raftery 1995), with PBF >10 considered strong support for the more parameterized model. Each analysis (two runs of four chains each) was run for 2×10^7 generations. Convergence was assessed using Gelman and Rubin's r statistic (Gelman *et al.* 1995). The lampropeltine species *Arizona elegans*, *Lampropeltis calligaster*, *Lampropeltis triangulum* and *Lampropeltis extenuata* were used as outgroups (File S1). Maximum-Likelihood (ML) analysis was performed using RAxMLv7.4.3 (Stamatakis 2006) with the same data set used for the Bayesian analysis. A codon position partitioned GTRMIX model was used, and 1000 nonparametric bootstrap replicates (Felsenstein 1985) were performed to assess node support. Bayesian posterior probabilities (Pp) greater than 95% are considered strong support for a clade, while bootstrap proportions (BS) greater than 0.7 are considered strong support (Hillis & Bull 1993; Felsenstein 2004).

Divergence time estimation

For divergence time estimation, we used relaxed-clock phylogenetics methods (Drummond *et al.* 2006) in the program BEAST version 1.4.8 (Drummond & Rambaut 2007). Divergence time estimation within species complexes is hampered by the necessity for a compromise between interspecific phylogenetic priors (tree priors) and intraspecific coalescent priors. It is inappropriate to combine inter- and intra-specific data in a single Bayesian divergence time analysis, as no prior can adequately account for both processes. We opt for the latter, combining our ingroup data set with a single outgroup, the sister taxon *Lampropeltis extenuata* (Pyron & Burbrink 2009a) and using coalescent priors for inferring divergence times using the entire population level data set. Although the inclusion of a nonconspecific outgroup taxon does not render all of the assumptions of the coalescent priors valid, this is a more conservative mode of analysis than attempting to date a large number of outgroups and a single representative from each lineage using only one mitochondrial gene.

We tested both increasing (logistic and exponential growth) and constant population size priors, which yielded similar results; we report the data from the constant population size prior. In addition, the inferred estimates are concordant with a multi-gene phylogenetic analysis of the tribe Lampropeltini (Pyron & Burbrink 2009a), suggesting that the estimated dates are robust to variation in priors. An uncorrelated lognormal tree prior, and lognormal fossil priors were used for divergence time estimation under the relaxed-clock model in BEAST (Drummond *et al.* 2006) using the same model

from the primary tree inference (3GTR + Γ + I). The divergence between *Lampropeltis extenuata* and *Lampropeltis getula* was constrained to have occurred during the Hemphillian, based on the fossils *Stilosoma (Lampropeltis) vetustum* and *Lampropeltis getula* known from the middle Hemphillian, late Miocene (Holman 2000). The mean of the lognormal distribution was 6.875 Ma (1.9278) with a standard deviation of 0.188, yielding a prior credible interval of 4.75–9.94 Ma. No zero-offsets were used. The analysis was run for 10 million generations, the first 2.5 million of which were discarded as burn-in. We assumed convergence when the effective sample size of the posterior probability distribution of all parameters was >200 (Drummond *et al.* 2006), calculated in Tracer v1.4 (Rambaut & Drummond 2007).

Historical biogeography

We used coalescent simulations in Mesquite version 2.5 (Maddison & Maddison 2008) to determine if the perceived Structured Model from the ML tree fits the data better than a widespread Fragmented Ancestor, based on potential stochastic variance in the tree structure and the observed number of deep coalescences (Knowles & Maddison 2002; Fig. 2). The Fragmented Ancestor model posits that all population divergences were in effect concurrent and resulted from the fragmentation of the widely distributed range of a common ancestor. The presence of phylogeographic structure under this model would be due to differential extinction of ancestral haplotypes among areas (Knowles 2001a, b; Carstens *et al.* 2005). The Structured Model suggests that a wide-ranging common ancestor originating across the Central US (Pyron & Burbrink 2009b) was first fragmented into two ancestral populations at the Mississippi River and then each of those was subsequently fragmented during colonization towards the western and eastern US, respectively (Fig. 1).

For coalescent simulations, we first estimated N_e for *L. getula* in each of the five geographically distinct areas determined from phylogeographic analyses using values for Θ calculated in the program MIGRATE-N version 2.4 (Beerli 2008) under the following parameters: 15 short chains for 200 000 generations and four long chains for two million generations with four adaptive heating chains, sampled every 20 generations following a burn-in of 10 000 generations. Maximum-likelihood estimates (MLE) were calculated three times to ensure convergence upon similar values for Θ . We converted Θ to N_e using the equation for maternally inherited mitochondrial DNA $\Theta = N_e\mu$, where $\mu = 3.0 \times 10^{-8}$ site/generation calculated in BEAST version 1.4.8 and a generation time of 3 years (Werler & Dixon 2000). We summed the estimates of N_e for all areas to calculate Total N_e and

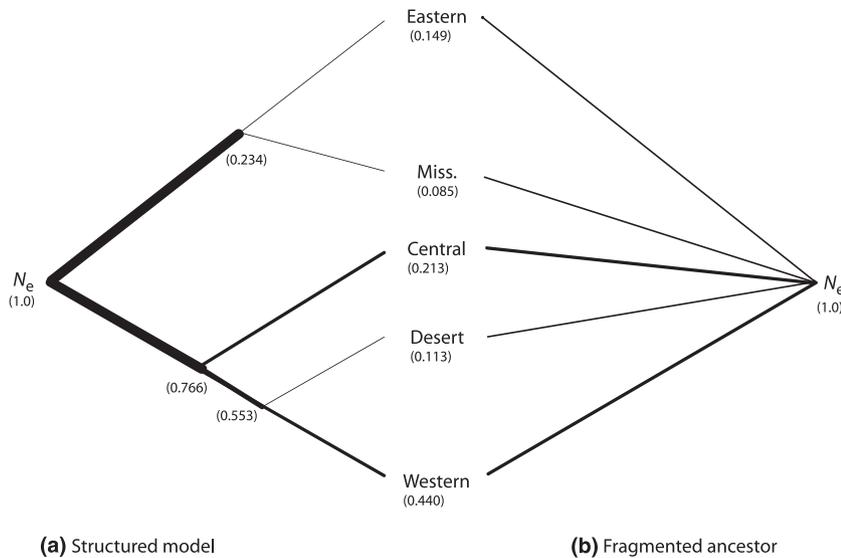


Fig. 2 Illustration of the hypothetical population ancestry models used for coalescent simulations: (a) Structured Model (the observed structure in the ML tree in Fig. 3) vs. (b) Widespread Ancestor (the fragmentation model). Values on the trees represent proportions of the total effective population size (see text).

scaled the branch widths of our hypothesized population trees using the proportion of Total N_e that each area comprised. Internal branches on the Structured Model were scaled such that all branch widths summed to Total N_e at any single point in time (Carstens *et al.* 2004; Shepard & Burbrink 2008, 2009; Fig. 2).

The method of counting the number of deep coalescences assumes that deep coalescent events are due to incomplete lineage sorting and not migration among populations (Knowles & Maddison 2002). In cases where the number of deep coalescences may be inflated by recent migration, it is important to account for migration in simulations to build null models that better reflect history under a given scenario (Shepard & Burbrink 2009). Using the MLE of Total N_e , we simulated 500 gene trees under a neutral coalescent process with migration on the Fragmented Ancestor model at a tree depth of 1 636 666 generations, which when based on a 3-year generation time is equivalent to 4.91 Myr (the approximate age estimated for the first divergence within *L. getula* using the fossil calibrated relaxed-clock phylogenetics method). To calculate the probability of migration per individual per generation for these simulations, we first multiplied values of M among adjacent populations (areas) calculated in MIGRATE-N version 2.4 (Beerli 2008) by the Θ of the receiving population to derive the number of immigrants per generation among pairs of adjacent populations. We divided these values by the estimated N_e of the source population to calculate the probability of emigration per individual per generation in the source population, and then calculated the mean of all population pairs to derive the average probability of migration per individual per generation.

We fit the simulated gene trees from the Fragmented Ancestor model into the Structured Model, calculated the number of deep gene coalescences (nDC) and built a distribution of nDC values. We then fit our reconstructed ML tree for *L. getula* to the Structured Model and calculated the nDC value. If this observed nDC falls below 95% of the distribution of nDC values calculated using the simulated gene trees (equivalent to one-tailed $P \leq 0.05$), then the Fragmented Ancestor model will be rejected in favour of the Structured Model. To calculate P values for the observed nDC values in these analyses, we fit the distribution of simulated nDC values to a normal distribution with the given mean and standard deviation (SD).

Niche modelling

Although the predicted habitat suitability from the ENM results is not an absolute prediction of the true fundamental or realized niche of an organism, it should provide a reasonable proxy and allows for statistical hypothesis testing regarding expressed niche preferences, at least with regard to the major environmental conditions experienced by the organisms (Warren *et al.* 2008; review in Kozak *et al.* 2008). To assess the impact of ecological niche on the formation and maintenance of lineage separation, we modelled the predicted suitable habitat of the inferred lineages of *L. getula* using maximum entropy methods (Elith *et al.* 2006; Phillips *et al.* 2006) in the program MAXENT version 3.2.19. The nineteen BIOCLIM variables from the WorldClim data set (Hijmans *et al.* 2005) were used at 30-s spatial resolution (~ 1 km).

Many of the BIOCLIM variables are highly correlated, and the relative contribution of each variable to the

model for each lineage is uncertain. Thus, we followed the protocol of Rissler & Apodaca (2007) and reduced the data set to 11 biologically informative variables, which are not significantly correlated across North America: BIO1–3, 7–9, and 15–19. In addition, we used the Level III Ecoregion designations for North America provided by the U.S. EPA and Commission for Environmental Cooperation. This layer, derived primarily from Omernik (1987), classifies North America into 182 distinct ecoregions based on biological and environmental ecosystem differentiation (Commission for Environmental Cooperation Working Group 1997). The ecoregions were trimmed to the same extent as the BIOCLIM variables and projected at 30-s spatial resolution.

To train the model, 733 georeferenced presence localities were obtained, comprising the samples used in our phylogenetic analysis and additional georeferenced museum records (File S2). The latter were obtained either through the public web interface of museum collections, or through the HerpNet database (<http://herpnet.org>). Records with GPS coordinates were used as is; all other records were georeferenced to the reported locality using the description provided in the record. Occurrences were assigned to lineages based on the circumscribed area as inferred from the primary phylogeographic analysis, and niche models were constructed for each lineage.

We used auto features in Maxent (Phillips *et al.* 2006), set the regularization multiplier to the default (1.0) and allowed the algorithm to run to convergence (threshold of 0.00001). The resulting niche predictions were projected onto a map of the U.S. in DIVA-GIS, with the minimum training presence criterion used as the binary threshold for predicted suitability. We attempted to assess qualitatively the biological niche differentiation between the lineages by determining which variables in the model contributed the greatest proportion of entropy to the model from the table given in the Maxent output. Overlap in predicted suitable area was calculated between adjacent lineages by counting the number of 30-s pixels predicted as suitable for both lineages. This was converted to area by multiplying by 0.86 (30 s of arc equals 0.93 km, thus a 30-s pixel equals 0.86 km²).

Niche differentiation

We assess differentiation in the predicted ENMs of the lineages using the newly developed niche equivalency methods of Warren *et al.* (2008). The program ENMTools uses two niche similarity metrics, Schoener's *D* (Schoener 1968) and the newly developed 'Warren *et al.*'s *I* (Warren *et al.* 2008). These statistics quantify predicted niche similarity, and range from 0 (no overlap) to 1 (identical niche models). We used the test of

niche equivalency in ENMTools, which evaluates equivalency between ENMs by comparing the observed values of *D* and *I* for the two models with a distribution of values of *D* and *I* based on randomized pseudoreplicates. This distribution is generated by randomly assigning occurrence points from both groups into one lineage or the other, simulating the potential overlap of a group of points occurring across a given geographical area (Warren *et al.* 2008). This allows for a one-tailed test of dissimilarity from random for two ENMs. As we are primarily reporting interactions between sister lineages, we did not employ the possible phylogenetic corrections for these analyses (Warren *et al.* 2008). We also did not employ the more stringent tests for niche equivalency given the available background, as no biological justification for defining the available background is evident for a single widespread species complex. We calculated the observed *D* and *I* values and simulated distributions of *D* and *I* using 100 pseudoreplicates for all pairwise comparisons of the inferred lineages; only those with relevance to our hypotheses are reported. Because of computational constraints, the pseudoreplicates niche models were inferred using the 11 BIOCLIM variables and the Level III Ecoregions projected at 2.5-min spatial resolution.

Results

The *cyt-b* gene sequenced for *Lampropeltis getula* and all outgroup taxa measured 1117 bp with no indels or stop codons in the reading frame for any sample. The sequences have been deposited on GenBank under the accession numbers FJ997648–FJ997848 (File S1). For the BI phylogenetic analysis, we chose the 3GTR + Γ + I model (PBF = 32.76). Gelman and Rubin's *r*-statistic (Gelman *et al.* 1995) was less than 1.001 for all parameters after a burn-in of 5×10^6 generations (split standard deviation among chains <0.01). Both analyses inferred five major lineages with generally strong support for both the lineages themselves and the relationships between them (Figs 1 and 3). The primary geographical lineages are as follows:

- 1 *Eastern*: A lineage comprising the kingsnakes of the eastern seaboard of the United States, from New Jersey to the Florida Keys and extending to the Apalachicola region in the Florida panhandle and southeast Alabama.
- 2 *Mississippi*: This lineage ranges through the greater Mississippi River drainage east of the Mississippi River, from southern Illinois east to Ohio and western West Virginia in the north, to the Tennessee and Alabama river drainages of Georgia and Alabama in the south.

- 3 *Central*: The Central lineage inhabits the Great Plains and Mississippi River valley west of the Mississippi River, from Iowa and Nebraska in the north to west-central Texas and the western Gulf Slope in the south, east to the Mississippi River.
- 4 *Desert*: The Desert lineage is found in the Chihuahuan Desert of west Texas, southern New Mexico (including the Rio Grande River Valley), extreme southeastern Arizona and eastern Mexico, along the Mexican Plateau. May also occur in north central Arizona (Fig. 4a).
- 5 *Western*: The Western lineage occurs west of the Rocky Mountains, from the southern Great Basin in Nevada and Utah, southern Oregon south to Baja California, and most of Sonora, Mexico, east to southeastern Arizona.

All lineages were strongly supported by both posterior probabilities ($P_p > 0.95$) and bootstrap proportions ($BS > 0.9$). The relationships among the lineages were also all supported at greater than 95% P_p and 90% BS , with the exception of the node subtending the Central lineage and the Western and Desert lineages, which received moderate support in the BI analysis (93% P_p) and weak support in the ML analysis (59% BS). Lineages of *L. getula* do not follow the currently designated subspecies taxonomy and appear to correspond to historical divergences at the Mississippi River (Western, Desert & Central vs. Eastern & Mississippi); the Rocky Mountains (Western & Desert vs. Central); the Cochise Filter Barrier (Western vs. Desert); and the Appalachian mountains/Chattahoochee River/Apalachicola River, here termed the Apalachee formation for ease of reference (Eastern vs. Mississippi). While some lineages (e.g. the Western and Eastern) exhibit strong concordance between the geographical mtDNA lineage and the currently described subspecies based on colour pattern (see Fig. 3; Blanchard 1921, Blaney 1977), others (e.g., the Central and Gulf lineages) do not. Thus, while morphological differentiation may be at least in part related to ecological divergence, the strength and underlying mechanisms of this pattern remain unclear.

Divergence dating and historical biogeography

Based on the prior distributions for the ages of the earliest known fossils of both taxa (Parmley & Holman 1995; Holman 2000), the dating analyses indicate that *L. getula* diverged from its sister taxon *Lampropeltis extenuata* during the Hemphillian of the late Miocene, ~6.54 Ma (95% Highest Posterior Density = 4.20–8.93 Ma). The earliest divergence occurred at the Mississippi River ~4.91 Ma (95% HPD = 2.63–7.32 Ma) during the late Hemphillian of the early Pliocene. This initial

divergence at the Mississippi River and an area of origin in the Central US corresponds with the area of origin for the tribe Lampropeltini, of which *L. getula* is a member (Pyron & Burbrink 2009a). Divergence between the western (Western & Desert) lineages and the Central lineage occurred during the Blancan of the early Pliocene (4.06 Ma; 95% HPD = 1.86–6.12 Ma). As fossils are known from Washington state dating to the Pliocene (Parmley & Walker 2003), we infer that this split occurred across the Rocky Mountains, and that the Cochise Filter Barrier divergence represents a subsequent west-to-east vicariance event. Thus, the parapatry of the Desert and Central lineages represents a zone of secondary contact. The Cochise Filter Barrier (2.16 Ma; 95% HPD = 1.11–3.44 Ma) and Apalachee (1.94 Ma; 95% HPD = 0.75–3.35 Ma) divergences occurred at approximately the same time at the Pliocene/Pleistocene boundary.

In terms of ancestral population structure, the Structured Model posits an initial divergence at the Mississippi River and subsequent divergences towards the West Coast (Central and Desert + Western, then Desert and Western clades) and the East Coast (Mississippi and Eastern clades). This structured tree was then tested against the Fragmented Ancestor model. Using Migrate-N version 2.4 (Beerli 2008), we calculated a maximum-likelihood estimate (MLE) of $\Theta_{\text{Total}} = 0.1034$ (95% CI: 0.06–0.161). The MLE of Θ_{Total} equates to a Total N_e of 3 446 666. Based on values of M and Θ from Migrate-N (Beerli 2008), we calculated a mean probability of migration per individual per generation of 3.351×10^{-6} , and used this value in coalescent simulations. The number of deep coalescent events (nDC) for our ML tree fit into the Structured Model was 54. This value was significantly less than results from the coalescent simulations under the Fragmented Ancestor tested against the Structured Model (mean nDC = 175.506, SD = 20.81539, $P = 2.586 \times 10^{-9}$). Therefore, all subsequent tests of niche used the structure from the ML tree representing the Structured model.

Niche modelling and equivalency

All predictions for each of the five lineages differed significantly from random (binomial test, $P < 0.00001$) and exhibited high sensitivity and specificity (AUC/ROC values > 0.95 for all lineages). The predicted distributions for each lineage closely matched their known extent (Figs 1, 3 and 4). The use of museum records, which precludes further discovery of between-lineage dispersal, may cause an underprediction in niche overlap. However, overlap in predicted suitability is observed between all adjacent lineages, and ranges from 0.0006% to 37% of the area of the lineages (Table 1;

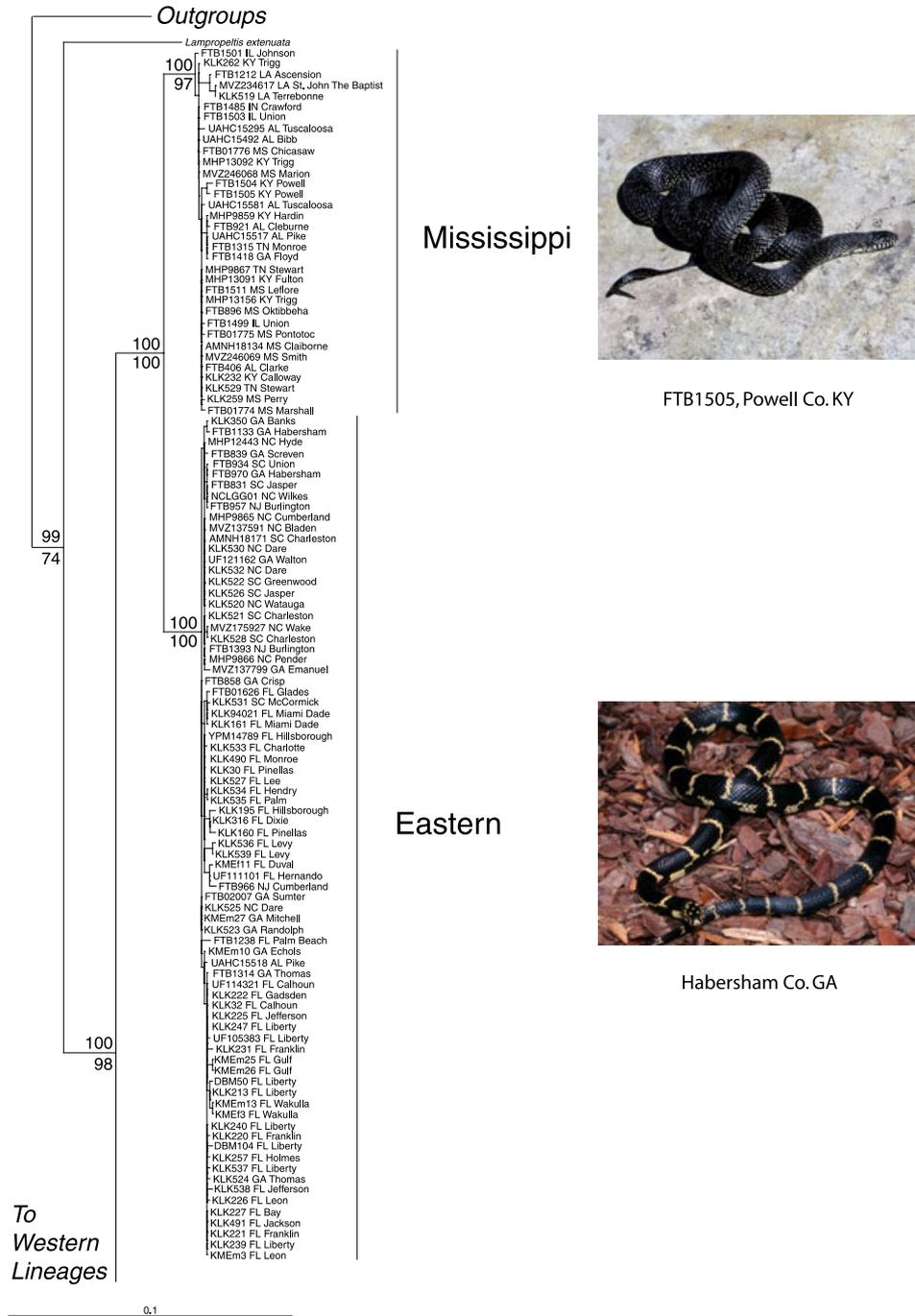


Fig. 3 The ML tree produced using 261 samples of *Lampropeltis getula* and four outgroups under a $3 \times$ GTRGAMMA model of evolution for the mitochondrial gene cytochrome b. Values above branches are the Posterior Probabilities from 1.5×10^7 post-burn-in samples; values below branches are the Bootstrap Proportion from 1000 nonparametric bootstrap replicates. Locations are given as State/Country and County/Parish, followed by collection numbers as follows: FT Burbrink (FTB), KL Krysko (KLK), Museum of Vertebrate Zoology (MVZ), University of Alabama Herpetological Collection (UAHC), Sternberg Museum (MHP), Yale Peabody Museum (YPM), KM Enge (KME), University of Florida (UF), DB Means (DBM), TJ Hibbitts, Texas A&M University (TJH, MTH & DL) Texas Natural History Collection (TNHC), Arkansas State University Museum of Vertebrate Zoology (ASUMZ), Illinois Natural History Survey (INHS), Texas Cooperative Wildlife Collection (TCWC), Donald B. Shepard (DBS), KJ Irwin (KJI), LJ Vitt (LJV), University of Texas, Arlington (UTA), DG Mulcahy (DGM), San Diego Field Station (SD Field), University of Nevada, Reno (UNR), San Diego Natural History Museum (SDSNH), California State University, Northridge (CSUN), Brad Moon, University of Louisiana, Lafayette (CG). Specimens labelled KLK, KME, UF and DBM were obtained from GenBank, from Krysko & Judd (2006). Photographs with voucher numbers are samples in the tree. Photographs for the Western and Central lineage are complements of SL & JT Collins, CNAH.

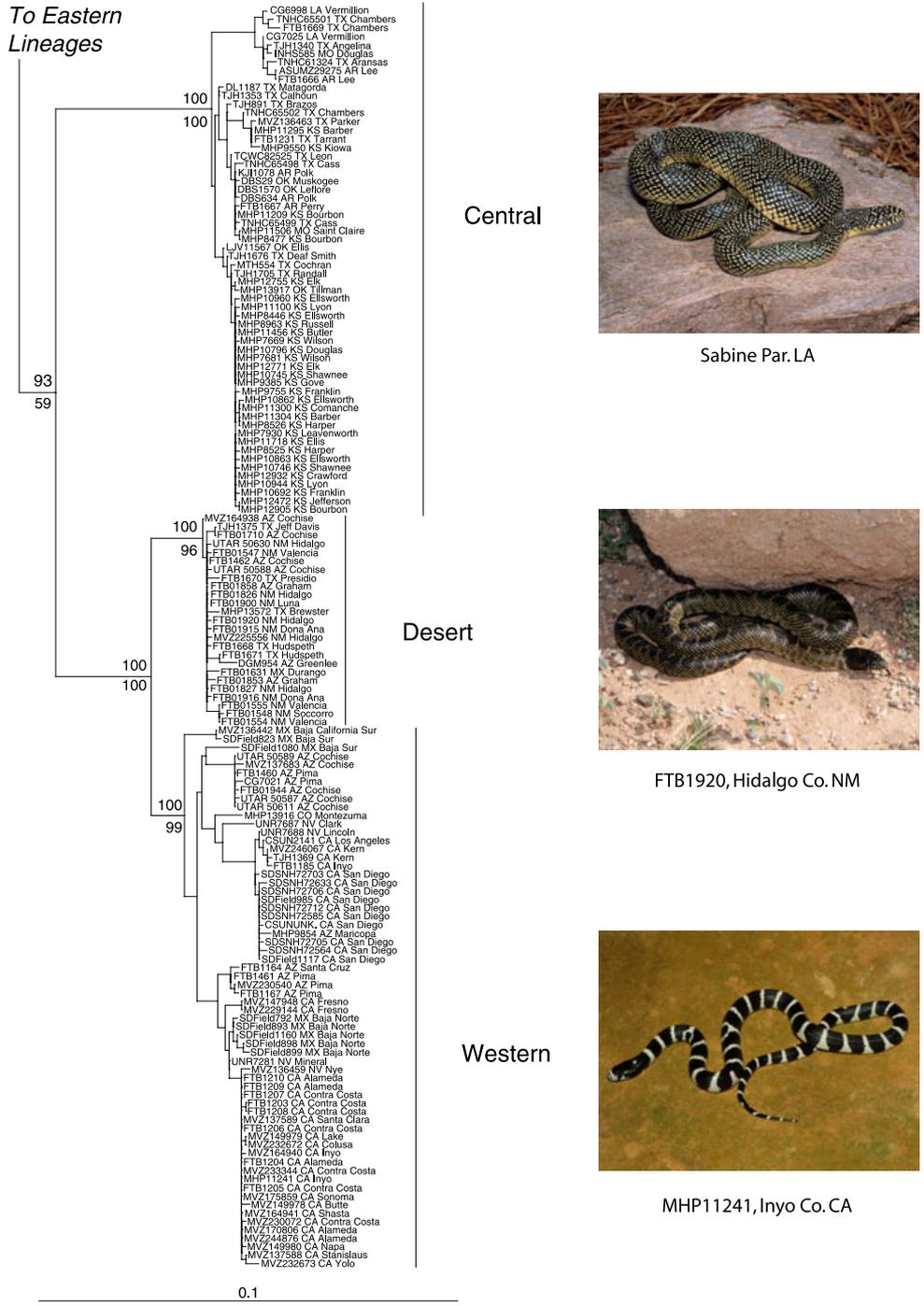


Fig. 3 Continued

Fig. 4). The variables with the greatest contribution to the models for each lineage were as follows: Western (BIO18: Precipitation of the Warmest Quarter; 44.7%), Desert [BIO2: Mean Diurnal Range (Temperature); 35.1%], Central (BIO1: Annual Mean Temperature; 41.9%), Mississippi (BIO17: Precipitation of the Driest Quarter; 62.6%) and Eastern (BIO7: Temperature Annual Range; 23.1%).

Schoener's *D* and Warren *et al.*'s *I* values for the pairwise comparisons of interest were significantly lower than expected from a random distribution (Table 1) for all comparisons except for the Western and Desert lineages, rejecting the null hypothesis of niche equivalency for all adjacent lineages. The Western and Desert lineages are not identical ($D = 0.65$, $I = 0.523$), but the interpredicted suitability does not differ significantly from the

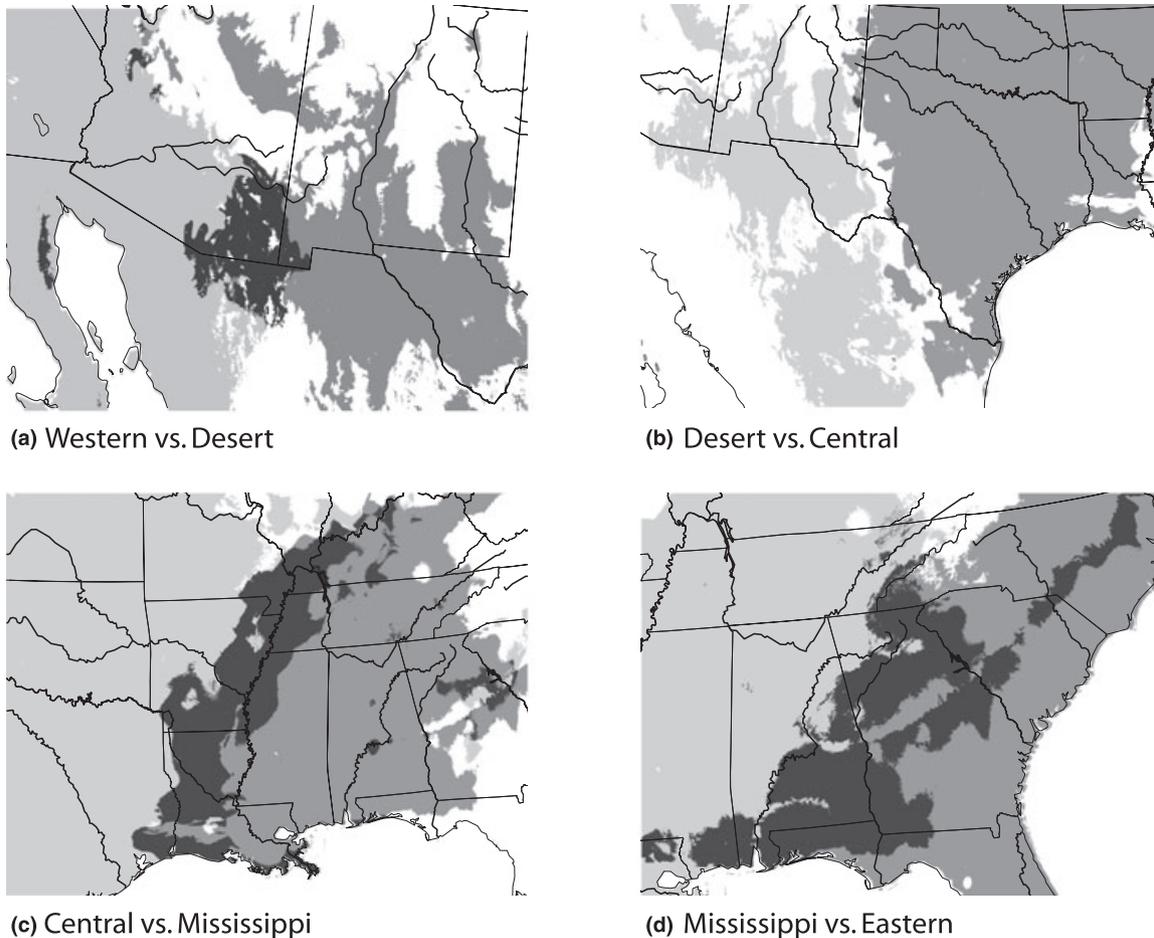


Fig. 4 Niche predictions for each inferred lineage at the points of primary geographical divergence and zones of secondary contact. Overlap between predicted suitable habitat is indicated by the darkest intermediate shade.

Table 1 Area of predicted habitat suitability for the five inferred lineages of *Lampropeltis getula* and niche overlap calculations for pairwise comparisons of adjacent clades

Lineage	Area	D (\bar{X} , SD, P)	I (\bar{X} , SD, P)
Eastern	823 590 km ²	—	—
Mississippi	1 085 876 km ²	—	—
Central	1 892 157 km ²	—	—
Desert	726 695 km ²	—	—
Western	1 225 441 km ²	—	—
E vs. M	279 184 km ²	0.265 (0.866, 0.031, <0.001)	0.523 (0.898, 0.022, <0.001)
C vs. M	304 064 km ²	0.172 (0.811, 0.025, <0.001)	0.441 (0.860, 0.017, <0.001)
C vs. D	1139 km ²	0.036 (0.312, 0.119, 0.01)	0.333 (0.542, 0.086, 0.008)
D vs. W	78 893 km ²	0.120 (0.275, 0.149, 0.150)	0.428 (0.502, 0.100, 0.227)

Area calculated as the total number of 30 arc second pixels predicted as suitable using a binary threshold, multiplied by 0.86 (30 s of arc = 0.86 km²). Niche overlap calculations for pairwise comparisons of adjacent clades. Overlap is the total area of the zone of predicted overlap in environmental suitability from the ENMs. Values in parentheses give the mean and standard deviation of the null distribution of D and I and the probability of the observed values from the niche equivalency test of Warren *et al.* (2008) implemented in ENMTools.

overlap between randomly assigned points. Given that these lineages are allo- or peripatric (Figs 1, 3 and 4), this is indicative of niche conservatism between these sister

lineages. At the zone of secondary contact between the Desert and Central lineages, a small amount of interpredicted suitability in the north appears to be

inhabited solely by the latter clade, whereas almost no overlap occurs along the western Gulf slope in the southern portion of the juncture, indicative of significant niche divergence (Table 1).

Discussion

Historical biogeography of the common kingsnake

The common ancestor of the kingsnake originated in the late Miocene and has since diverged to form five lineages across major geographical barriers in North America. The initial divergence at the Mississippi River (~5.0 Ma) is a pattern, which has been documented in numerous species of both plants and animals (Burbrink *et al.* 2000, 2008; Howes *et al.* 2006; Soltis *et al.* 2006; Lemmon *et al.* 2007). Subsequently, three lineages diverged westwards at ~4.0 Ma (Central and Western/Desert) and 2.1 Ma (Western and Desert) and two eastwards ~1.9 Ma (Eastern and Mississippi). Divergences at the Cochise Filter Barrier (~2.1 Ma) in the West and the Apalachee formation (~1.9 Ma) in the East occurred more recently, and at a similar time near the Pliocene/Pleistocene boundary. This suggests that the congruent patterns of lineage divergence observed at these features may be the result of similar responses to physiographic and environmental shifts during the late-Pliocene and early-Pleistocene (Soltis *et al.* 2006; Castoe *et al.* 2007).

At the Cochise Filter Barrier, the separation of the Sonoran and Chihuahuan desert provinces during the late Pliocene (Morafka 1977) has been widely implicated in the formation of geographical lineages across the western deserts as refugia formed on either side of the continental divide during the Pliocene and Pleistocene (Jaeger *et al.* 2005; Smith & Farrell 2005; Devitt 2006; Castoe *et al.* 2007; Mulcahy 2007). The observed divergence in the Western and Desert lineages of the kingsnake at ~2.1 Ma is contemporaneous with the formation of Sonoran/Chihuahuan/Mojave clades in cactus longhorn beetles (*Monoleima armatum*; Smith & Farrell 2005), western diamondback rattlesnakes (*Crotalus atrox*; Castoe *et al.* 2007) and desert spiny lizards (*Sceloporus magister*; Leaché & Mulcahy 2007). Although few studies have dated divergences at the Apalachee formation, most implicate the late-Pliocene and Pleistocene formation of glacial refugia in the southern Appalachians and Florida in the separation of lineages (Burbrink *et al.* 2000; Soltis *et al.* 2006; Pauly *et al.* 2007).

All five lineages occupy geographically distinct habitats according to niche modelling results and exhibit varying areas of predicted overlap. The ENMs of the sister Eastern and Mississippi lineages are found to be significantly different based on randomization tests, although the areas of predicted overlap are also associ-

ated with apparent sympatry of the lineages (Figs 1, 3 and 4). Predicted areas of overlap between the sister Western and Desert lineages are also associated with an area of sympatry (Figs 1, 3 and 4), although the ENMs for these lineages do not differ significantly, which suggests a role for niche conservatism promoting speciation across the Cochise Filter Barrier (Fig. 1). The predicted overlap between the significantly different ENMs of Central and Mississippi lineages is not associated with widespread sympatry of the lineages, suggesting that the Mississippi river is a strong barrier to dispersal. The near-total lack of predicted overlap between the Central and Desert lineages suggests a role for niche divergence in promoting, or at least maintaining, allopatric population segregation along an environmental gradient.

Conservatism and divergence in ecological niche

Rather than finding that a single process of niche evolution played a dominant role, all three modes of niche-barrier interaction are associated with lineage formation in the kingsnake. We find (i) niche conservatism at the Cochise Filter Barrier; (ii) niche divergence at the Apalachee Formation and the Mississippi River; and (iii) niche divergence at the ecological transition between the adjacent Central and Desert/Western clades, which appears to lack a physical barrier. The sister lineage pairs (Western/Desert and Eastern/Mississippi) both appear to have separated along axes of precipitation and temperature, although the particular aspects of those variables are not consistent across the lineages. For a species occupying a range the width of a continent, it is not surprising to find a range of patterns suggesting different modes of lineage divergence with respect to ecological niche. Niche conservatism has been identified as the mechanism responsible for the formation of many organisms such as species of plethodontid salamanders in temperate areas (Kozak & Wiens 2006; Shepard & Burbrink 2008, 2009). In contrast, niche divergence has been found to promote diversification in organisms such as tropical salamanders occurring along elevational gradients (Kozak & Wiens 2007) and ecological gradients in other ectothermic vertebrates (Graham *et al.* 2004; Raxworthy *et al.* 2007). However, to our knowledge, a role for both mechanisms in diversification and lineage formation in a single species has not been shown.

Although niche conservatism appears to be present between some lineages in *Lampropeltis getula*, our results show that ecological niches have not been broadly conserved among lineages to a degree where adjacent lineages share identical ENMs. This suggests that environmental preferences are labile even on recent timescales, and species may evolve significant

differences even between recently diverged sister lineage pairs as natural selection acts on populations in ecologically heterogeneous habitats (Wiens 2004). Indeed, ENM results predict almost complete ecological separation between the peripatric Western and Central lineages (Figs 1 and 4).

The indication from these results is that niche differentiation is based on the dominant ecological feature of the local environment. Thus, dry season precipitation exerts the strongest influence on the Mississippi clade, which inhabits the mesic Mississippi River drainage, while annual temperature most strongly affects the Central lineage, which occurs as far north as Nebraska, in areas subject to extreme winters. However, even the nondifferentiated Western and Desert lineages show influence from different, presumably locally adapted variables: daily temperature range for the Desert lineage and rainy-season precipitation for the Western lineage. Ultimately, while niche conservatism and divergence may both influence lineage formation, the particular niche differences between any given lineages may simply reflect historical contingencies rather than a unified pattern of ecological influence.

Although niches must always be conserved in the sense that descendant populations will inhabit similar geographical areas or ecological niches as their immediate ancestors (i.e. Wiens & Graham 2005; Losos 2008a, b; Wiens 2008), our results provide an important perspective on the influence of niche conservatism on speciation. The detection of niche equivalency between the sister Western and Desert lineages, which are distributed across a putative climatic barrier, is indicative of the classic scenario for speciation through niche conservatism across a continuous landscape (Wiens 2004). However, the ecological divergence between the remaining lineages illustrates a more subtle point about niche conservatism. The maintenance of lineages in geographically distinct areas must be due, at least in part, to the conservation of niche preferences through natural selection against individuals that disperse out of the current niche (e.g. Holt & Gaines 1992; Wiens 2004). Indeed, phylogenetic niche conservatism has been shown to influence the continental distributions of many organisms (Ricklefs & Latham 1992; Wiens *et al.* 2006, 2009; Pyron & Burbrink 2009b). While niche conservatism may exert a powerful influence on the distribution of species, it is still possible for lineages to exhibit divergence in environmental preferences on short evolutionary timescales.

Conclusions

We find evidence that both niche conservatism and niche divergence have played roles in promoting and main-

taining divergence between lineages of *L. getula*, as well as zones of secondary contact between lineages. While conservation of ancestral ecological conditions was detected between some lineages, our results indicate that ecological niche, or at least predicted habitat suitability based on expressed environmental preferences, is labile even on recent evolutionary timescales, and that niche evolution and divergence may occur rapidly even between sister lineages. Moreover, all of these processes are shown to have occurred and are associated with lineage formation in the common kingsnake. While authors have recently argued about the basic definition of niche conservatism and the extent to which niches are conserved (Losos 2008a, b), it is apparent that it is not whether niches are conserved that is important for phylogenetic studies, but the extent to which niches are conserved and the impact that this has on our understanding of the processes which influence speciation (Wiens & Graham 2005; Wiens 2008). The evolutionary history of organisms, particularly of recently diverged species complexes, is probably a mixture of the inertial tendency of populations to maintain their current niche and the action of natural selection on populations, which differ in habitat across ecological landscapes.

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Supporting information

Additional supporting information may be found in the online version of this article.

File S1 Collection localities for all specimens of *Lampropeltis getula* used in this study, as well as GenBank accession numbers for cytochrome *b* sequences for all samples.

File S2 733 georeferenced presence localities comprising the samples used in our phylogenetic analysis and additional georeferenced museum records.

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