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Exploring Chihuahuan Desert diversification in the gray-banded kingsnake, *Lampropeltis alterna* (Serpentes: Colubridae)

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ABSTRACT

Within many biomes, the cause of phylogeographic structure remains unknown even across regions throughout North America, including within the biodiverse Chihuahuan Desert. For example, little is known about population structure or the timing of diversification of Chihuahuan endemics. This is due largely to the lack of population genomic studies within this region. We generated ultra-conserved element data for the gray-banded kingsnake (*Lampropeltis alterna*) to investigate lineage divergence and historical demography across the Chihuahuan Desert. We found three unique lineages corresponding to the Trans-Pecos and Mapimian biogeographic regions of the Chihuahuan Desert, and a distinct population in the Sierra Madre Occidental. Using several mutation rates to calibrate the timing of divergence among these lineages, we show that lineage divergence likely occurred during the Pleistocene, which indicates that careful consideration needs to be used when applying mutation rates to ultra-conserved elements. We suggest that biogeographic provinces within the Chihuahuan Desert may have served as allopatric refugia during climatic fluctuations of the Quaternary. This work serves as an important template for further testing biogeographic hypotheses within the region.

1. Introduction

Population structure and ultimately speciation are influenced by historical events, landscape features, and biotic influences. In many cases, shared processes affect the population structure of many disparate taxa (Carnaval et al., 2009; Gehara et al., 2017), demonstrating that common large-scale features of biomes often have unique effects on gene flow. While processes related to lineage formation within temperate biomes have been dissected for many plant and animal species (Soltis et al., 2006; Shafer et al., 2010), other regions remain largely unexplored, and therefore, important features of lineage divergence, such as timing of divergence and the basic drivers of diversification, remain unknown.

Although the Chihuahuan Desert is the largest warm desert in North America and is both ecologically diverse and species rich (Hernández et al., 2001; Olson and Dinerstein, 2002), few studies have examined processes that structure populations of endemic taxa in this region. Broad-scale phylogoeographic patterns across arid North America have demonstrated the distinctiveness of this region's biodiversity from adjacent arid biomes (e.g., Zink et al., 2001; Riddle and Hafner, 2006; Myers et al., 2017). The Chihuahuan Desert has been delineated into three biogeographic subprovinces (Trans-Pecos, Mapimian, and Saladan; Fig. 1), based on shared species distributions (Morafka, 1977). Population genetic structure in several taxa is congruent with these subprovinces (e.g., Scheinvar et al., 2017), which also largely correspond to the Trans-Pecos, Coahuilan, and Zacatecan subregions delineated by Hafner and Riddle (2005). Studies investigating the origins of population structure of Chihuahuan Desert taxa have produced dates that range broadly from the late Neogene to the Quaternary (e.g., Sosa et al., 2009; Bryson et al., 2012; Castellanos-Morales et al., 2016; Loera et al., 2017), suggesting that species are likely responding to unique features of this biome. For example, divergences that date to the

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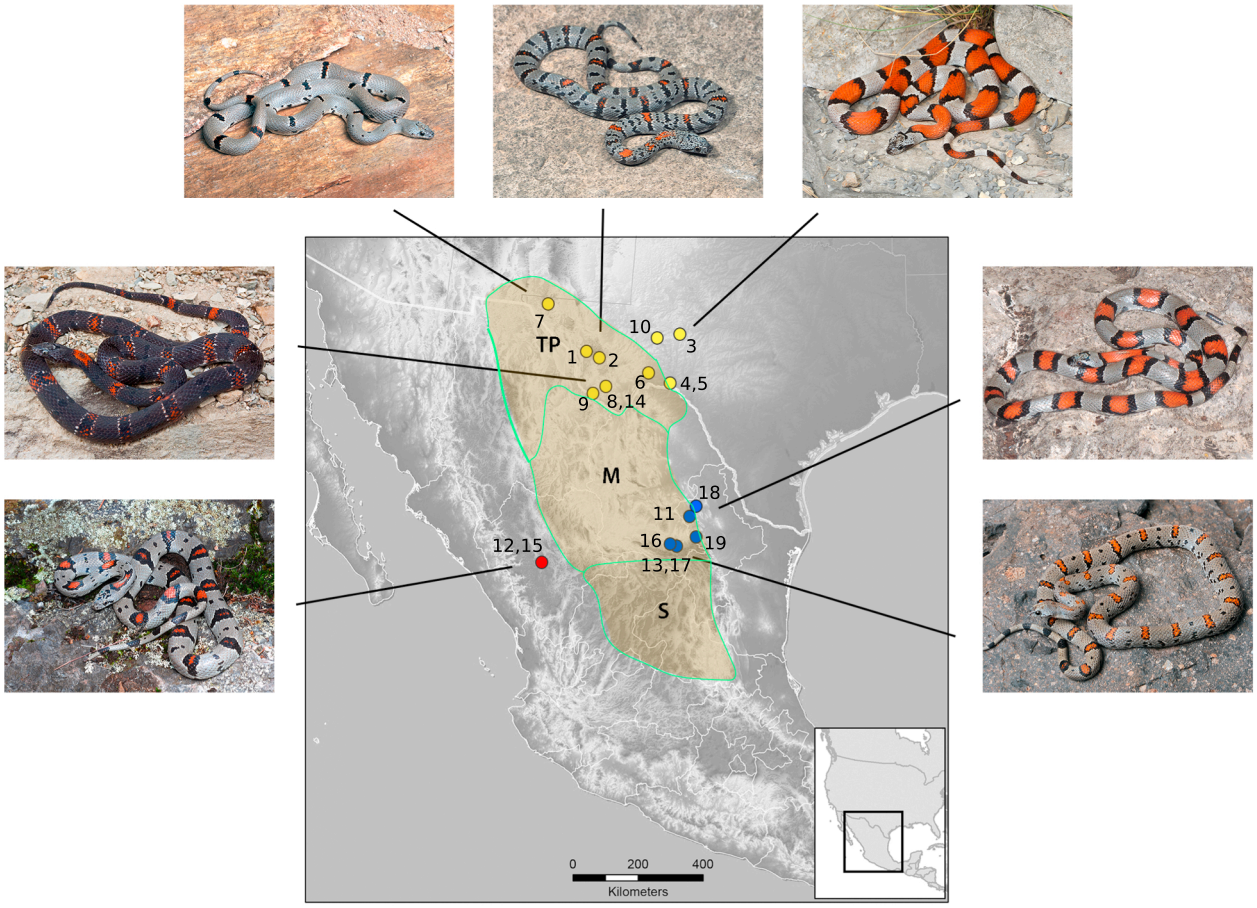

Fig. 1. Sampling localities of *Lampropeltis alterna* included within this study. Colors of each symbol denote population membership. Outlined in green and shaded in tan is the approximate distribution of the Chihuahuan Desert, divided into the three subprovinces of Morafka (1977); TP = Trans-Pecos, M = Mapimian; and S = Saladan. Around the map are images illustrating phenotypic variation found within *L. alterna*. Additional locality info and collection numbers are listed in the Supporting information.

Quaternary are usually associated with glacial and climatic cycles (Ayoub and Riechert, 2004; Jaeger et al., 2005; Wilson and Pitts, 2012; Loera et al., 2017) and post-Pleistocene colonization from southern refugia (Wells and Hunziker, 1976; Wells, 1977). Neogene divergences appear to be linked to orogenic events that promoted the expansion of arid habitats in western North America (Wilson and Pitts, 2010; Wood et al., 2013) resulting in geographic population structure predating Quaternary climatic oscillations (Van Devender and Burgess, 1985; Van Devender, 1990; Steadman et al., 1994; Thompson and Anderson, 2000; Metcalfe, 2006).

Snakes in temperate North America have provided important examples of how populations are structured given climate and habitat changes as well as ancient geographic features (Burbrink and Castoe, 2009). In particular, kingsnakes in the genus *Lampropeltis* present strong phylogeographic structure consistent with geography and ecological gradients across many habitat types and have diversified during the major climate cycles of the Pleistocene (e.g., Pyron and Burbrink, 2009; Burbrink et al., 2011; Myers et al., 2013; Ruane et al., 2014; McKelvy and Burbrink, 2017). As an endemic Chihuahuan Desert taxon, the gray-banded kingsnake (*L. alterna*) is distributed across the mountainous regions of the Trans-Pecos and Mapimian subprovinces of the Chihuahuan Desert, inhabiting a range of habitats from xeric desert scrub to subhumid wooded uplands (Fig. 1; Garstka, 1982; Bryson et al., 2007; Hansen and Salmon, 2017). While this taxon is polymorphic in color and pattern (Fig. 1), nothing is known of its population genetic structure or historical demography. Previous mitochondrial DNA (mtDNA) gene-tree analyses have demonstrated that *L. alterna* is polyphyletic with respect to *L. mexicana* and *L. gentilis* (Bryson et al., 2007). This polyphyly is the result of mitochondrial introgression from *L. gentilis* into *L. alterna* and not deep coalescence of the mtDNA genome (Ruane et al., 2014). Despite this mitochondrial introgression, nuclear DNA suggest little to no gene flow between *L. alterna* and *L. gentilis*, and support *L. alterna* as a distinct genetic cluster that is not sister to *L. gentilis* in species-tree analyses (Burbrink and Gehara, 2018; Ruane et al., 2014), further demonstrating that mitochondrial introgression is the result of historical rather than contemporary processes. Although *L. alterna* was not the focus of these previous studies, it was demonstrated that understanding processes related to divergence and historical demography requires information from many loci, and that the mtDNA genome will be largely uninformative for population level studies of *L. alterna*.

To better understand processes of lineage divergence in *L. alterna* and historical demographics in the Chihuahuan Desert, we use sequence capture to generate a genomic dataset of ultra-conserved element loci. With these data, we assess spatial genetic diversity, asking if predefined biogeographic areas within the Chihuahuan Desert show genetically distinct populations. We then use several mutation rates and secondary calibrations to estimate the timing of diversification to assess the importance of geologic events of the Neogene versus Quaternary climatic cycles in driving lineage divergence.

2. Methods and materials

2.1. Sampling, library prep, and sequencing

We sampled 19 *L. alterna* from across its distribution in the US and

several regions of Mexico (Fig. 1, Supplemental Materials). Samples were included from both the Trans-Pecos and Mapimian subprovinces of the Chihuahuan Desert as well as the only known population from the lower eastern foothills of the Sierra Madre Occidental. Although these tissue samples span the entire known distribution of *L. alterna*, we were unable to obtain samples from a few regions in Mexico. We included *L. zonata* as an outgroup taxon because it is the sister group to *L. alterna* (Chen et al., 2017). We extracted genomic DNA from tissues and shed skins using Qiagen DNeasy Blood and Tissue Kits (Qiagen Inc.). Extractions were quantified using a Qubit 2.0 fluorometer (Life Technologies, Inc.) and sent to RAPiD Genomics (Gainesville, FL, USA) for ultraconserved element (UCE) sequence capture and sequencing. Each pool was enriched using a set of 5472 custom-designed probes (MYbaits, MYcroarray, Inc.) targeting 5060 UCE loci (Faircloth et al., 2012) following an open-source protocol (available at www.ultraconserved.org). Pooled libraries were sent to the University of Florida ICBR Facility for 100 bp paired-end sequencing on an Illumina HiSeq 3000. Using dual-indexed barcodes, multiple clean-ups and quality control steps, the amount of reads that mapped to impossible index combinations was very low (average index hopping rate = 0.2%), alleviating concerns raised in Sinha et al. (2017).

2.2. UCE assembly and alignment

To reduce the possibility that the reads from more diverged samples either would not map or would map incorrectly, we assembled UCEs from each sample independently using a modified version of the Phyluce pipeline (Faircloth, 2015). Briefly, we first used the VelvetOptimiser wrapper to de novo assemble each sample with Velvet (v.1.2.1; Zerbino and Birney, 2008). Our lower hash value was set to 65 and our upper value to 75. The optimization function used for Velvet's coverage cutoff was set to the total number of base pairs in all contigs ('tbp'). All other parameters were set to the default. After de novo assembly, we used the Phyluce scripts 'phyluce_assembly_get_match_counts' and 'phyluce_assembly_get_fastas_from_match_counts' to match each sample's contigs to the reference probes. Next, we used the Burrows-Wheeler Aligner (BWA; v. 0.7.12; Li and Durbin, 2009) to map the reads to the de novo assembled contigs (each sample being unique). After mapping, PCR duplicates were removed with Picard Tools (v.1.119; https://broadinstitute.github.io/picard/). Then we used The Genome Analysis Toolkit (GATK; v3.6-0; McKenna et al., 2010) to call, realign, and mask indels, call and annotate snps, and perform read-backed phasing, all in accordance with the standard Phyluce pipeline. A phased, sample-specific FASTA was created using 'add_phased_snps_to_seqs_filter.py' from the seqcap_pop pipeline (Harvey et al., 2016). Lastly, we combined all samples into multi-FASTAs (one per UCE) using a custom Perl script. These were aligned using MUSCLE (v3.8.31; Edgar, 2004) with default settings, then trimmed with a custom Perl script such that there were no missing sites at the ends of alignments. Custom scripts used in preparing our data for analysis are available upon request from the authors.

From these aligned and trimmed UCE series, two data sets were constructed and used for downstream analyses. The first was a complete matrix of UCE loci that were represented in all *L. alterna* and *L. zonata* samples and therefore had no missing data (this data set will be referred to as the 'complete dataset' throughout). In the second dataset, loci were conditioned on being present in the outgroup taxon (*L. zonata*) and allowed for missing data within the *L. alterna* samples (hereafter, 'relaxed dataset').

2.3. Population structure and isolation by distance

Using the complete dataset of UCE loci, a single, random nucleotide polymorphism (SNP) was extracted from each UCE for use in population structure analyses. Two complementary methods that do not make explicit assumptions about HW equilibrium or linkage disequilibrium (McVean, 2009) were used to determine the number of genetic clusters and assign individuals to clusters. The first was the find.clusters function of the *adegenet* R package (Jombart, 2008); this method consists of running successive K-means clustering with increasing numbers of K, after transforming the data using PCA. For each number of K, a statistical measure of goodness of fit defined as Bayesian information criterion (BIC), is computed to find the optimal K value. These results were visualized with the DAPC function of *adegenet*. Second, we implemented sNMF (Frichot et al., 2014) of the LEA R package (Frichot and François, 2015). This method estimates admixture coefficients via sparse non-negative matrix factorization algorithms, and produces admixture coefficient outputs. For this method, the number of K is statistically defined by cross-entropy scores. Here we tested K values ranging from 1 to 5 with 100 repetitions each.

We also assessed relationships among each sampled individual by concatenating the complete dataset and estimating a phylogenetic tree in RAxML v 8.2.10 (Stamatakis, 2006). This analysis assumes bifurcation, which may not be biologically realistic for population level sampling, especially for phased data. However, it is likely a useful exploratory tool to test for clustering of individuals (Harrington et al., 2017). We performed a rapid bootstrapping analysis, rooting the tree with *L. zonata* and using the GTR GAMMA model of sequence evolution.

Isolation-by-distance (IBD) is a common feature of spatial genetic variation within species and can confound population structuring analyses (Meirmans, 2012). We therefore tested for IBD using redundancy analyses, a constrained ordination method (Legendre and Fortin, 2010) using modified R scripts from Myers et al. (2017). We chose to implement redundancy analyses because this method tests for associations between genetic divergence and geographic distances while circumventing statistical problems that can arise from distance measures using Mantel tests (Kierepka and Latch, 2015). A genetic distance matrix was generated using a random SNP per UCE locus from the complete dataset using the dist.genpop function of *adegenet*. This genetic distance matrix was used as a response variable, where latitude and longitude between sampled localities (19 unique localities) were the explanatory variables. We also conducted this analysis within two of the populations inferred from the above population structuring analyses (11 and 6 unique localities; note that the third population contained two individuals with the same locality data, not enough for tests of correlation). This analysis computes an r^2 value and uses ANOVA to assess significance in a pattern of IBD.

2.4. Species tree inference

To explicitly test whether the genetic clusters determined by DAPC and sNMF represented independently evolving lineages that should be used for demographic inference and to infer the topology of a species tree, we implemented Bayesian Phylogenetics and Phylogeography v.3.3 (BPP; Yang and Rannala, 2010, 2014). This method uses the multispecies coalescent model to infer species trees in a Bayesian framework, accounting for incomplete lineage sorting and gene-tree species-tree conflict (Yang and Rannala, 2010; Rannala and Yang, 2013). In this analysis, we used the complete dataset, including the outgroup taxon (*L. zonata*), and ran the joint unguided species delimitation and species tree inference. Both population size (θ) and root divergence time of the species tree (τ_0) were assigned gamma distribution priors $G(\alpha, \beta)$. We implemented three combinations of priors: large ancestral population size with deep divergence time, $\theta \sim G(1, 10)$ and $\tau_0 \sim G(1, 10)$; small ancestral population size and shallow divergences, $\theta \sim G(2, 2000)$ and $\tau_0 \sim G(2, 2000)$; and large ancestral population sizes and recent divergences, $\theta \sim G(1, 10)$ and $\tau_0 \sim G(2, 2000)$. We ran two independent analyses for each set of priors for 500,000 generations, after a burn-in period of 10,000 generations, and a sampling frequency of one every five generations.

2.5. Demographic history and parameter estimation

To estimate the demographic history of this taxon, we used the full likelihood approach implemented in G-PhoCS (Gronau et al., 2011). G-PhoCS uses the multi-species coalescent to estimate divergence times and effective population sizes (N_e) from multilocus sequence data and models gene flow between populations given user-defined migration bands (Gronau et al., 2011). Gamma distributions G(α, β) were used to specify prior distributions on theta ($\theta = 4N_e\mu$, where μ is the mutation per nucleotide site per generation), τ (species divergence time, $T_{DIV} = \tau/\mu$), and m_{sx} (the proportion of individuals in population *x* that arrive via migration from population *s* per generation). We ran G-PhoCS analyses under two different priors (τ – θ [1, 300] and [1, 30]) that represented both shallow and deep phylogeographic divergences and smaller and larger N_e (Oswald et al., 2017). Each of these prior settings was run twice to ensure consistency among runs. For this analysis, we used the larger, relaxed dataset of UCEs that included the outgroup taxon, *L. zonata*. The topology was fixed given the results of the concatenated RAxML and the unguided species tree estimation of BPP. Two migration bands were specified, with gene flow allowed between the Trans-Pecos and Mapimian populations, and between the Mapimian and Durango populations. Each analysis was run for 1,000,000 generations, sampling every 500 iterations, we checked for convergence in the MCMC runs in Tracer v1.6 (Rambaut et al., 2014).

A mutation rate or secondary calibration is required to convert the parameter estimates into time before present and effective population sizes. However, mutation rates are often imprecisely estimated, unknown for most non-model organisms, and unknown for loci that are commonly the targets of sequence capture probes like UCEs (Oswald et al., 2016). Therefore, we used two different rates that have recently been applied to population genomic studies of snakes that used RADseq data (7.26×10^{-9} mutations/site/generation assuming a generation length of 3.3 years from Harrington et al., 2017; 2.5×10^{-8} mutations/site/generation assuming ~5.0 years/generation from Sovic et al., 2016; Gibbs et al., 2018). These mutation rates were estimated from mammals and lizards (Nachman and Crowell, 2000; Kumar and Subramanian, 2002; Gottscho et al., 2014) respectively, and neither of these rates are based on UCEs, which may have lower rates of substitution (Katzman et al., 2007). Therefore, we also used previous divergence time estimates from coalescent based analyses between *L. alterna* and *L. zonata* to fix the root of these analyses, allowing us to estimate average UCE mutation rates, which were then used to estimate divergence times within *L. alterna* (3.1 mya, Ruane et al., 2014; 3.31 mya Chen et al., 2017).

3. Results

3.1. Sampling and UCE data sets

From the 19 individual *L. alterna* specimens sampled, a total of 403 UCE loci were recovered in the complete data matrix where missing data were not allowed. Alternatively, 2339 loci were recovered in the data matrix when missing individuals across loci were permitted, which also included the outgroup taxon *L. zonata*.

3.2. Population structure

Both DAPC and sNMF supported the presence of three genetic clusters with the same sample membership, across the distribution of *L. alterna* (Fig. 2; Supplemental Materials). These results corresponded to a population in the Trans-Pecos province of Texas and New Mexico (Trans-Pecos population), a population in northeastern Mexico corresponding to the Mapimian region (Mapimian population), and a population from the Sierra Madre Occidental of western Durango (Durango population). In the supplemental materials we provided *Q*-matrix bar plots from sNMF for each K-value from 2 to 5. Results from the concatenated maximum likelihood tree recovered three lineages, which corresponded to the populations identified in both genetic clustering analyses (Supplemental Materials). Redundancy analyses demonstrated that geographic distance was a strong predictor of genetic distances between sampling localities across the distribution of this taxon (adjusted $r^2 = 0.45$, p-value < 0.001). IBD was also significant within the Trans-Pecos population (adjusted $r^2 = 0.13$, p-value < 0.01), but not within the Mapimian population (adjusted $r^2 = 0.13$, p-value > 0.1).

3.3. Species tree inference and demographic history

BPP analyses supported all three genetic clusters within *L. alterna* as distinct lineages. These results were consistent among different prior combinations used and replicate runs across these priors (Table 1). The topology of the species tree was less well supported. However, most analyses recovered the relationships to be *L. zonata* sister to a monophyletic *L. alterna* clade consisting of the Durango population, which was sister to the Trans-Pecos, Mapimian group (posterior probability of 0.74 across all combinations of priors; Table 1).

Parameter estimates of τ and θ from G-PhoCS analyses were robust to both priors used and results were consistent across replicate runs. Rates of migration were low, estimated at ~0.1 migrant per generation between all populations in which migration bands were inferred. However, we note that it has been demonstrated that posterior estimates of migration rates inferred by G-PhoCS are sensitive to prior distributions (Gronau et al., 2011; Smith et al., 2014), therefore we interpreted this parameter estimate with care. The use of mutation rate greatly influenced the inferred divergence times and effective population sizes (Table 2). For example, the two rates previously used in snake phylogeography studies differed by an order of magnitude, and suggested divergence times between *L. zonata* and *L. alterna* at 43,300 ya (mutation rate of 7.62×10^{-9}; Harrington et al., 2017) and 13,200 ya (mutation rate of 2.5×10^{-8}; Sovic et al., 2016). When fixing this divergence time based on previously published studies (see methods), we estimated the average UCE mutation rate to be 1.06×10^{-10} (from divergence times in Ruane et al., 2014) and 9.97×10^{-11} (from divergence times in Chen et al., 2017). These rates estimated the divergence time between Trans Pecos and Mapimian populations at 1.41 mya and 1.5 mya respectively, and between the western Durango and all other populations at 2.42 mya and 2.58 mya respectively (Table 2). Estimated effective population sizes were similarly affected, where point estimates differ by orders of magnitude based on assumed mutation rates, which also differed by an order of magnitude (Table 2).

4. Discussion

Our results show that *Lampropeltis alterna* is composed of three distinct geographic lineages within the Chihuahuan Desert. Two of these divergent lineages have geographic distributions restricted to biogeographic provinces within the Chihuahuan Desert, specifically the northern Trans-Pecos region and the central Mapimian province (Fig. 1). The timing of divergence events between these lineages occurred within the Pleistocene (1.41–1.5 mya and 2.42–2.58 mya; Table 2) when applying secondary calibrations, suggesting that climatic oscillations that characterized this time period may have been important in structuring genetic diversity within this taxon. All three lineages are supported as being distinct using molecular species delimitation methods (Table 1). These populations have diverged within well-defined biogeographic regions (Fig. 1) that are separated by the Rio Grande and Rio Conchos, suggesting these rivers may have been important drivers of phylogeographic diversification within the Chihuahuan Desert during the Pleistocene. In addition, geographic distance is an important variable in explaining genetic diversity across the distribution of gray-banded kingsnakes.

Divergence with gene flow is common, even in groups that have diverged long ago or that are ecologically very different (Niemiller

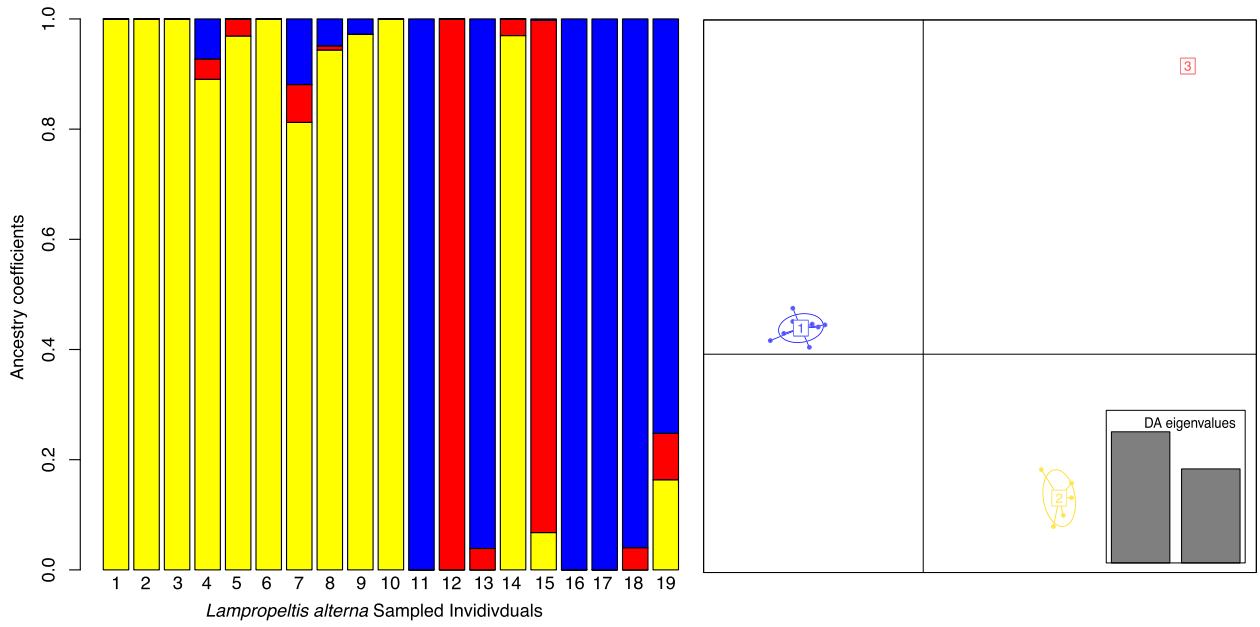

Fig. 2. Results from population structure analyses of *Lampropletis alterna*. Left, admixture proportions for each individual sample inferred from sNMF, numbers along the x-axis correspond to the numbered localities in Fig. 1. Right, scatterplot of DAPC demonstrating the three distinct genetic clusters.

Table 1
Results from species tree inference and species delimitation of *Lampropeltis alterna* from BPP under three different sets of priors.

Priors	Best tree topology	Posterior probability for tree	Species delimitation
$\theta \sim G(2, 2000)$, $\tau_0 \sim G(2, 2000)$	(*zonata*, (Durango, (Trans-Pecos, Mapimian)))	0.99	(*zonata*, (Durango, (Trans-Pecos, Mapimian)'#1.0') '#1.0') '#1.0'
$\theta \sim G(1, 10)$, $\tau_0 \sim G(2, 2000)$	((Durango, *zonata*), (Trans-Pecos, Mapimian))	0.54	((Durango, *zonata*) '#1.0', (Trans-Pecos, Mapimian) '#1.0') '#1.0'
$\theta \sim G(1, 10)$, $\tau_0 \sim G(1, 10)$	(*zonata*, (Durango, (Trans-Pecos, Mapimian)))	0.69	(*zonata*, (Durango, (Trans-Pecos, Mapimian) '#0.99') '#1.0') '#1.0'

et al., 2008; Ellegren et al., 2012; Leaché et al., 2013). Here we show little gene flow among gray-banded kingsnake lineages that diverged in the late Quaternary. This suggests that these lineages are reproductively isolated and likely formed in allopatry. Analyzing additional genomic data is necessary to test realistic models of geographic speciation (Yang et al., 2017). Under a model of strict allopatry, it is expected that genes responsible for the evolution of reproductive isolation will have the same divergence as non-functional regions of the genome, whereas under a model of speciation with gene flow, the divergences of these speciation genes will predate the divergence of the rest of the genome (Ting et al., 2001; Yang et al., 2017).

Population genetic structure in *L. alterna* corresponds to distinct Chihuahuan Desert biogeographic provinces. Our results suggest that the climatic fluctuations of the Quaternary were important in structuring genetic diversity and therefore these biogeographic provinces may have acted as Pleistocene refugia. This geographic pattern of genetic structure has been observed in another Chihuahuan endemic, *Agave lechuguilla* (Scheinvar et al., 2017). Within *Agave lechuguilla*, however, the estimated timing of lineage divergence at 4.4 mya predates our divergence-time estimates within the gray-banded kingsnake (Table 2). This suggests that while similar spatial patterns of divergence may be observed in both plants and animals of the Chihuahuan Desert, the timing and causes of divergence may be different. Alternatively, differences in divergence dates between kingsnake populations and *Agave* populations could also be due to estimating time using sequence data given variance in molecular clocks among lineages or the influence of secondary calibrations (Graur and Martin, 2004; Schenk, 2016). Future studies could focus on whether similar community-wide patterns and timing of divergence within this desert exist.

We estimated divergence times using both previously published mutation rate estimates (Nachman and Crowell, 2000; Kumar and Subramanian, 2002; Gottscho et al., 2014) and secondary calibrations (Ruane et al., 2014; Chen et al., 2017). These previously published rates were likely too fast for substitutions from UCE loci given that the timing of divergence between *L. alterna* and *L. zonata* has been estimated numerous times at > 3 mya based on both fossil-calibrated phylogenomic and multi-locus species tree estimates (Chen et al., 2017; Ruane et al., 2014), which is two orders of magnitude greater than times inferred using RADseq mutation rates (Table 2). Because of this, we are more confident in the parameter estimates based on the secondary calibrations (based on Ruane et al., 2014; Chen et al., 2017, note that the posterior probabilities of parameter estimates for these two calibrations broadly overlap and therefore are statistically indistinguishable; Table 2), placing divergence times within the Pleistocene. Because the discrepancies between our estimates are large, we advocate exploring several mutation rates or calibrations to better understand potential error around divergence times and effective population sizes. It is important for studies to use several different rates for the same data set and present all estimates to better illustrate uncertainty in divergence time estimates (herein; Myers et al., 2017).

Implementing a multispecies coalescent model to test competing species delimitation hypotheses strongly supports the presence of three distinct lineages within *L. alterna*. Although our genomic samples span the known geographic distribution of *L. alterna* (Hansen and Salmon, 2017), it remains unknown if the inferred genetic clusters are geographically isolated. For example, no *L. alterna* samples exist from central Coahuila and eastern Durango. Although *L. alterna* has not been found in central Coahuila north of the Cuatrociénegas Basin or from

Table 2
Divergence times and effective population among inferred lineages of *Lampropeltis alterna* sizes given different rates of mutation or calibration times.

Reference	N_e Trans-Pecos	N_e Mapimian	N_e Durango	N_e ancestral (Trans-Pecos, Mapimian)	N_e ancestral ((Trans-Pecos, Mapimian), Durango)	Divergence time (Trans-Pecos, Mapimian)	Divergence time ((Trans-Pecos, Mapimian), Durango)	Divergence time (*L. alterna* complex, *L. zonata*)	Mutation rate
Harrington et al. (2017)	21,500 (19,400–23,400)	28,500 (25,100–31,700)	9,000 (7,500–10,700)	13,400 (8,100–18,700)	9,100 (1,900–16,200)	19.7 kya (19.4–22.3)	33.8 kya (29.2–37.7)	43.3 kya (37.4–49.5)	Fixed–7.62e − 9
Sovic et al. (2016)	26,200 (23,700–28,500)	34,800 (30,600–38,600)	11,000 (9,200–13,100)	16,400 (9,900–22,800)	11,100 (2,300–19,700)	6 kya (5.9–6.8)	10.3 kya (8.9–11.5)	13.2 kya (11.4–15.1)	Fixed–2.5e − 8
Ruane et al. (2014)	1,548,000 (1,397,000–1,680,000)	2,050,000 (1,804,000–2,276,000)	646,000 (542,000–772,000)	965,000 (584,000–1,344,000)	654,000 (136,000–1,162,000)	1.41 mya (1.39–1.6)	2.42 mya (2.09–2.7)	Fixed–3.1 mya	1.06e − 10
Chen et al. (2017)	1,645,000 (1,486,000–1,787,000)	2,180,000 (1,919,000–2,420,000)	687,000 (577,000–812,000)	1,026,000 (621,000–1,429,000)	695,000 (144,000–1,235,000)	1.5 mya (1.48–1.7)	2.58 mya (2.23–2.88)	Fixed–3.31 mya	9.97e − 11

Coahuila and eastern Durango, suitable habitat does exist (Fig. 1). Our data exhibit strong patterns of isolation-by-distance, which is pervasive across vertebrates (Wang et al., 2013; Pelletier and Carstens, 2018) and can confound defining population structure with genomic data alone (Meirmans, 2012; Bradburd et al., 2018). Simulations have shown that the processes that give rise to a pattern of IBD (e.g., limited dispersal or philopatry) can result in speciation in the absence of natural selection, geographic barriers to gene flow, or ecological gradients (Hoelzer et al., 2008; Baptestini et al., 2013). Although our genetic data support the existence of three reproductively isolated lineages of *L. alterna*, we refrain from suggesting taxonomic changes. If there are intermediate populations and the signal of IBD is consistent across the species distribution, then it remains possible that these inferences are the result of arbitrarily dissected clinal variation. Finding divergent lineages of *L. alterna*, while perhaps not unexpected given the high levels of cursorily cryptic diversity within other *Lampropeltis* species complexes (Pyron and Burbrink, 2009; Burbrink et al., 2011; Myers et al., 2013; Ruane et al., 2014; McKelvy and Burbrink, 2017), underscores how little species-level diversity has been documented to date.

The work presented here demonstrates that biogeographic provinces within the Chihuahuan Desert harbor genetically distinct populations of *L. alterna*. These lineages diversified during the Pleistocene, a period of extreme climatic fluctuations. This suggests that the Trans-Pecos and Mapimian regions would have served as allopatric refugia during periods of glacial maxima. It is likely that additional phylogenomic studies of endemic species within the Chihuahuan Desert will show similar patterns of population structure with divergence times within the Quaternary.

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Data Archiving

The data generated during the current study are available from the NCBI Sequence Read Archive (Accession: PRJNA497917) and the alignments used in this study are available on Dryad (doi: 10.5061/dryad.f10h6p7).

Appendix A. Supplementary material

sNMF plots for K = 2, 4, and 5.; Table of sampling locality info; Results from concatenated gene-tree analysis; Cross entropy scores from sNMF and BIC scores from adegenet analyses. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2018.10.031.

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