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Testing the geographical dimensions of genetic diversity following range expansion in a North American snake

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Spatial and demographic expansion can alter patterns of genetic variation and have predictable spatial and temporal consequences. Two-dimensional range expansion should result in genetic variation that is correlated with the geographical axis of expansion. Notably, populations across the range of a geographically widespread species may experience expansion and contraction events asynchronously. We tested for the genetic consequences of range expansion in the flat-headed snake, *Tantilla gracilis*, which inhabits the North American Great Plains and has few barriers to terrestrial dispersal. We sequenced mitochondrial DNA (mtDNA) and nuclear DNA from across the range of *T. gracilis*, inferred phylogenies and constructed haplotype networks. We observed multiple geographically circumscribed mtDNA clades that varied greatly in their spatial extent, but little range-wide variation in nuclear DNA. Mitochondrial sequence variation was negatively associated with latitude across their geographical range, the expected pattern of northward expansion. Finally, the three largest mtDNA clades exhibited varying demographic patterns, with only one being consistent with recent expansion. Despite substantial mtDNA cladogenesis, the predicted signature of northern range expansion was still detectable in *T. gracilis*. Our results bolster the notion that post-Pleistocene range expansion was a dominant force shaping the genetic diversity of vertebrates in central North America.

ADDITIONAL KEYWORDS: Great Plains – landscape genetics – phylogeography – population expansion– *Tantilla gracilis*.

INTRODUCTION

Geographical ranges can both expand and contract during the evolutionary history of a species, and these range dynamics can impact the evolution of descendant lineages. Range contraction can isolate populations, reducing genetic diversity within and promoting genetic divergence among populations (Arenas *et al.*, 2012). Range expansion can lead to low levels of genetic diversity at range fronts and therefore has the potential to create widespread, genetically homogeneous groups of individuals (Excoffier *et al.*, 2009). Crucially, understanding how range expansion and contraction can impact genetic diversity has important implications for delimiting species (Streicher *et al.*, 2012, 2016), detecting introgression (Moseley *et al.*, 2015) and predicting evolutionary responses to ecological change (Pearson & Dawson, 2003).

The expansion and contraction of populations and geographical ranges can fragment and coalesce the distribution of species episodically. Contractions can feature depleted genetic diversity attributable to population bottlenecks, although rapid range contractions may preserve greater genetic diversity than slow range contractions (Widmer & Lexer, 2001; Arenas *et al.*, 2012). If the ecological or climatic conditions that caused the contraction are reversed, then populations can experience demographic and geographical range expansion (Provan & Bennett, 2008). This spatial and demographic expansion has predictable consequences for molecular evolution within a species

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(Provan & Bennett, 2008; Excoffier *et al.*, 2009; Slatkin & Excoffier, 2012). Range expansion decreases genetic diversity owing to founder effects at the range front, with the most common alleles being over-represented and rarer alleles under-represented (Excoffier *et al.*, 2009). Importantly, populations across the geographical range may experience varying dynamics of demographic expansion, leading to complex patterns of genetic diversity across the geographical range.

Many species with subtropical and temperate distributions in the Northern Hemisphere show a pattern of recent expansions associated with global warming during the Quaternary (Prentice *et al.*, 1991; Hewitt, 2000; Swenson & Howard, 2005; Streicher *et al.*, 2012; Adams & Hadly, 2013). This pattern is particularly notable in North America, where the recession of the Laurentide and Cordilleran ice sheets presumably increased the amount of suitable habitat for temperate biota at northern latitudes (Hundertmark *et al.*, 2003; Kuchta & Tan, 2004; Emerson *et al.*, 2010). This relatively rapid expansion of the geographical range of species in the Northern Hemisphere that followed glacial recession has been implicated in ubiquitous geographical patterns of genetic diversity, where substantial genetic diversity is harboured at southern latitudes and is depleted in the northern portions of their geographical range (Hewitt, 2000; Swenson & Howard, 2005).

We studied genetic diversity across the geographical range of the North American flat-headed snake, *Tantilla gracilis*. This small, semifossorial snake is found throughout the south-central USA west of the Mississippi River and north of Mexico (Dixon, 2000; Werler & Dixon, 2000; Ernst & Ernst, 2003; Lemos-Espinal *et al.*, 2004), a region where many animal species exhibit the molecular signature of recent range and population expansion (Hamilton *et al.*, 2011; Streicher *et al.*, 2012; Moseley *et al.*, 2015; Burbrink *et al.*, 2016), including snakes (Cox & Chippindale, 2014; Ruane *et al.*, 2015; Streicher *et al.*, 2016; McKelvy & Burbrink, 2017). We combined range-wide geographical sampling, multilocus molecular phylogenetics and analyses of molecular diversity to study genetic variation in the flat-headed snake, which has probably been influenced by both refugial dynamics and range expansion. Our aims were as follows: (1) to characterize population and phylogenetic structure across the geographical range; (2) to assess geographical patterns of genetic diversity; and (3) to test for the molecular signature of northern range expansion within and among clades. We predicted that past population increase would lead to a genetic legacy of reduced genetic diversity, particularly at the northern segment of the geographical range, which would be the expansion front under a scenario of glacial recession and northern range expansion. We also predicted that demographic expansion would have a geographical dimension, and thus that there should be a negative relationship between latitude and genetic diversity if the species underwent recent range expansion to the north.

MATERIAL AND METHODS

Study system and geographical sampling

Flat-headed snakes are distributed from Missouri and Louisiana in the east to the Texas panhandle and the Mexican state of Coahuila in the west (Fig. 1). Snakes were collected across this geographical range by turning rocks in appropriate habitat from 2008 to 2011 (Fig. 2). We preserved muscle, liver or skin tissue in lysis buffer, 95% ethanol or an RNA-preserving buffer. Although some specimens were sampled for tissues and released, most specimens were fixed in 10% formalin and fluid preserved in 70% ethanol. Specimens were deposited in the Amphibian and Reptile Diversity Research Center at the University of Texas at Arlington. Additional tissue samples were obtained from the Louisiana Museum of Natural History at Louisiana State University and The Texas Natural History Collection at the University of Texas. In addition, we sequenced DNA from four other *Tantilla* species (*T. coronata*, *T. nigriceps*, *T. planiceps* and *T. wilcoxi*) and obtained data for an additional species (*T. relicta*) from an unpublished dissertation (Holm, 2008) and data for two outgroup species (*Conopsis biserialis* and *Coluber constrictor*) from GenBank (Table 1).

Molecular analysis

DNA was isolated using Qiagen DNeasy kits (Qiagen, Valencia, CA, USA) following standard protocols. We amplified and sequenced two mitochondrial loci, totalling 1351 bp: a fragment of NADH dehydrogenase subunit 4 and flanking tRNAs (685 bp); and cytochrome *b* (666 bp). We amplified and sequenced three nuclear genes, totalling 1160 bp: synuclein alpha interacting protein (*SNCAIP*; 261 bp); recombination activating gene (*RAG-1*; 478 bp); and brain derived neurotropin factor (*BDNF*; 421 bp). Mitochondrial loci were amplified with 2 min denaturation at 95 °C, with 35 cycles of denaturation (95 °C for 30 s), annealing (50 °C for 30 s) and extension (72 °C for 1 min), and a final 10 min extension at 72 °C. Nuclear loci were amplified with a touchdown procedure using an initial 2 min denaturation step at 95 °C followed by 40 cycles of denaturation at 95 °C (30 s) and extension at 72 °C (1 min), with annealing for 30 s at temperatures from 56 to 50 °C that stepped down by 2 °C every 10 min,

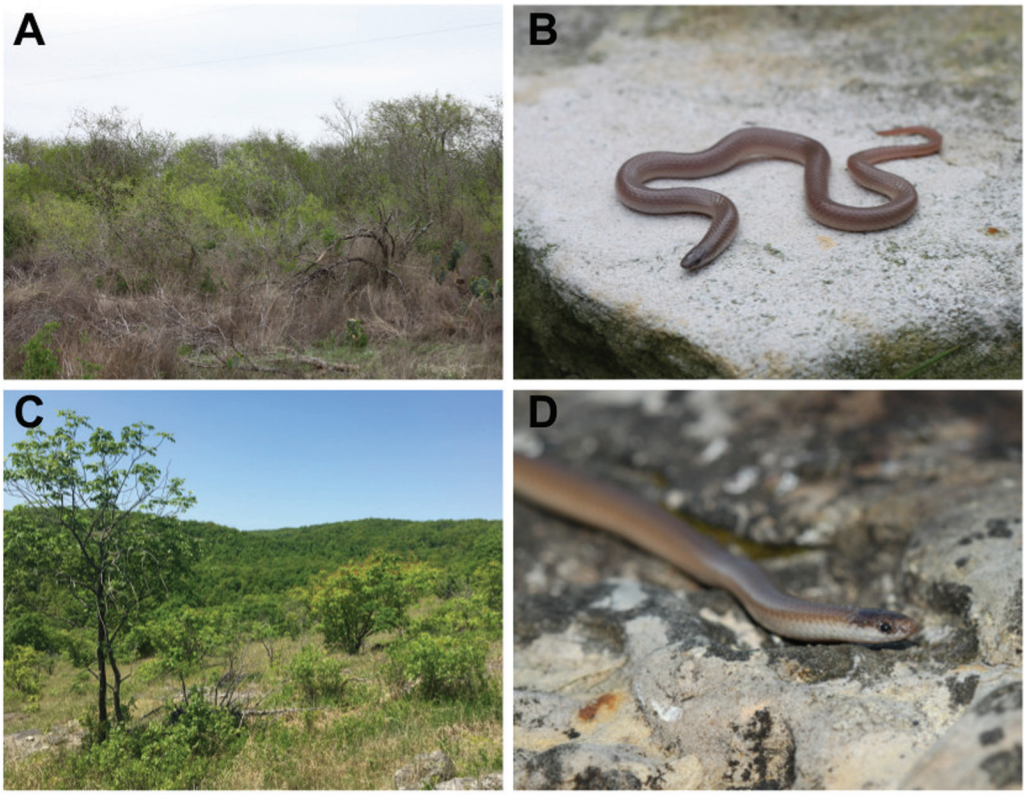

Figure 1. *Tantilla gracilis* and habitat from the southern extent of their geographical range in Hidalgo County, Texas, USA (A, B) and the northeastern extent of their range in Taney County, Missouri, USA (C, D).

with a final extension for 10 min at 72 °C. We used gel electrophoresis in 1% agarose to confirm amplification, and cleaned PCR products for sequencing using the ExoSAP-IT kit (United States Biochemical). The BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) was used for cycle sequencing, with the products precipitated using an ethanol/sodium acetate/EDTA method and rehydrated in formamide (Hi-Di). Samples were analysed using an ABI PRISM 3100xl Genetic Analyzer in the Genomics Core Facility at the University of Texas at Arlington. Chromatograms were edited using Sequencher (Genes Code Corp.) and aligned using the ClustalW algorithm (Larkin *et al.*, 2007) implemented in MEGA v.5.2 (Tamura *et al.*, 2011), using default parameters.

Phylogenetic reconstruction and genetic networks

Mitochondrial data were analysed with a variety of phylogenetic methods. We used MEGA v.5.2 (Tamura *et al.*, 2011) to test aligned sequences for the most appropriate model of molecular evolution among mitochondrial genes and codon positions. The GTR+G model ranked in the top three models for each group based on the Akaike information criterion and Bayesian information criterion scores; therefore, we chose this model for the subsequent analyses. We used MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) with default priors to perform Bayesian tree searches on concatenated mitochondrial loci. We ran multiple tree searches with varying priors and partition schemes without major changes to topology. We used Markov chain Monte Carlo searches for 5 000 000 generations, sampling every 500 generations, using three heated chains and one cold chain, and checked stationarity using parameter outputs in MrBayes (Huelsenbeck & Ronquist, 2001) and the program TRACER v.1.4 (Drummond & Rambaut, 2007). For this and all subsequent analyses, we discarded 50% of the trees as burn-in. We used RAxML v.7.0.3 (Stamatakis, 2006) to conduct maximum likelihood (ML) analysis using 100 ML tree searches and 10 000 bootstrap replicates on the best-scoring topology to obtain nodal support values. Finally, we analysed these data using maximum parsimony in MEGA v.5.2 (Tamura *et al.*, 2011), with nodal support assessed by 1000 bootstrap replicates.

We constructed genetic networks using the TCS method (Clement *et al.*, 2002) in the program POPART (www.popart.otago.ac.nz) for both mitochondrial DNA

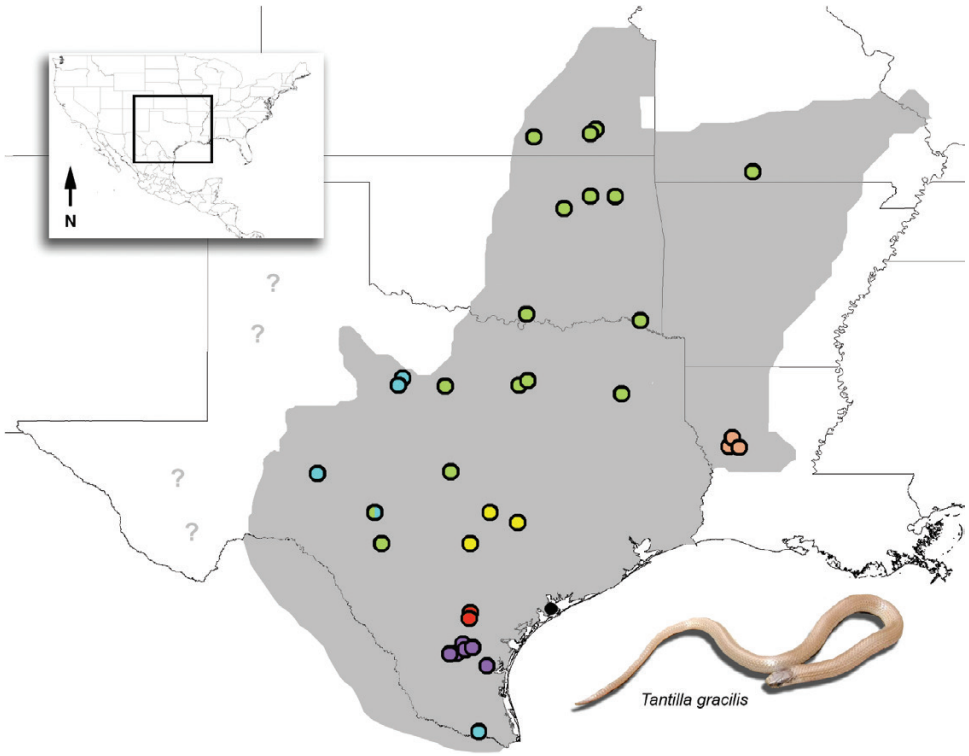

Figure 2. Geographical range of *Tantilla gracilis*. Each coloured circle indicates a locality with a tissue sample in the present study. The black circle is the type locality of *T. gracilis*. The colour of the circles on the maps indicates mitochondrial clade membership (see Fig. 3).

Table 1. Specimen IDs and locality of tissues in this study

Tissue ID	Species	Country	State	County	GPS N	GPS W
CJF4247	*Tantilla gracilis*	USA	TX	Tarrant	32.7011	–97.1633
CLC029	*Tantilla gracilis*	USA	TX	Shackleford	32.6699	–99.4544
CLC030	*Tantilla gracilis*	USA	TX	Shackleford	32.6699	–99.4544
CLC040	*Tantilla gracilis*	USA	OK	Creek	35.9962	–96.3334
CLC042	*Tantilla gracilis*	USA	OK	Creek	35.9962	–96.3334
CLC047	*Tantilla gracilis*	USA	OK	Love	34.0112	–97.0454
CLC121	*Tantilla gracilis*	USA	TX	Hidalgo	26.1214	–97.9610
CLC135	*Tantilla gracilis*	USA	TX	Palo Pinto	32.6534	–98.5768
CLC156	*Tantilla gracilis*	USA	OK	Love	34.0112	–97.0455
CLC157	*Tantilla gracilis*	USA	OK	Love	34.0112	–97.0455
CLC171	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC172	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC173	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC174	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC175	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC176	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC177	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC178	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC179	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC180	*Tantilla gracilis*	USA	TX	Crockett	31.0236	–101.0011
CLC331	*Tantilla gracilis*	USA	KS	Cowley	37.3487	–96.8833
CLC332	*Tantilla gracilis*	USA	KS	Cowley	37.3487	–96.8833

Table 1: Contiued

Tissue ID	Species	Country	State	County	GPS N	GPS W
CLC333	*Tantilla gracilis*	USA	KS	Cowley	37.3487	–96.8833
CLC345	*Tantilla gracilis*	USA	Texas	Hidalgo	26.1214	–97.9610
CLC376	*Tantilla gracilis*	USA	TX	Kleberg	27.3910	–97.7824
CLC448	*Tantilla gracilis*	USA	OK	Tulsa	36.2208	–95.8294
CLC449	*Tantilla gracilis*	USA	OK	Tulsa	36.2208	–95.8294
CLC450	*Tantilla gracilis*	USA	OK	Tulsa	36.2208	–95.8294
CLC461	*Tantilla gracilis*	USA	KS	Wilson	37.4715	–95.7578
CLC462	*Tantilla gracilis*	USA	KS	Wilson	37.4715	–95.7578
CLC463	*Tantilla gracilis*	USA	KS	Wilson	37.4715	–95.7578
CLC468	*Tantilla gracilis*	USA	KS	Wilson	37.4440	–95.8104
CLC469	*Tantilla gracilis*	USA	KS	Wilson	37.4440	–95.8104
CLC484	*Tantilla gracilis*	USA	MO	Taney	36.6673	–92.8058
CLC517	*Tantilla gracilis*	USA	TX	Bastrop	30.0899	–97.1988
CLC518	*Tantilla gracilis*	USA	TX	Bastrop	30.0899	–97.1988
CLC538	*Tantilla gracilis*	USA	TX	Live Oak	28.3239	–98.1136
CLC539	*Tantilla gracilis*	USA	TX	Live Oak	28.3239	–98.1136
CLC624	*Tantilla gracilis*	USA	TX	Duval	27.7685	–98.2472
CLC630	*Tantilla gracilis*	USA	TX	Duval	27.7724	–98.2353
CLC634	*Tantilla gracilis*	USA	TX	Duval	27.7513	–98.2281
CLC646	*Tantilla gracilis*	USA	TX	Duval	27.5928	–98.4673
CLC647	*Tantilla gracilis*	USA	TX	Duval	27.5928	–98.4673
CLC648	*Tantilla gracilis*	USA	TX	Duval	27.5928	–98.4673
CLC649	*Tantilla gracilis*	USA	TX	Duval	27.5928	–98.4673
CLC650	*Tantilla gracilis*	USA	TX	Duval	27.5928	–98.4673
CLC685	*Tantilla gracilis*	USA	TX	Jim Wells	27.7246	–98.0795
CLC686	*Tantilla gracilis*	USA	TX	Jim Wells	27.7246	–98.0795
CLC694	*Tantilla gracilis*	USA	TX	Duval	27.6030	–98.4135
CLC712	*Tantilla gracilis*	USA	TX	Live Oak	28.3401	–98.1032
CLC713	*Tantilla gracilis*	USA	TX	Live Oak	28.3401	–98.1032
CLC717	*Tantilla gracilis*	USA	TX	Duval	27.5928	–98.4673
EACP205	*Tantilla gracilis*	USA	TX	Dallas	32.7566	–96.9987
EACP69	*Tantilla gracilis*	USA	TX	Dallas	32.7566	–96.9987
JWS059	*Tantilla gracilis*	USA	TX	Shackleford	32.7947	–99.3699
JWS281	*Tantilla gracilis*	USA	TX	Kimble	30.2906	–99.9108
JWS282	*Tantilla gracilis*	USA	TX	Kimble	30.2906	–99.9108
CLC543	*Tantilla gracilis*	USA	TX	Smith	32.4942	–95.2637
LSUMZ H19766	*Tantilla gracilis*	USA	LA	Natchitoches	31.5274	–93.2054
LSUMZ H21019	*Tantilla gracilis*	USA	LA	Vernon	31.3429	–93.2494
LSUMZ H2470	*Tantilla gracilis*	USA	LA	Natchitoches	31.4886	–93.0291
LSUMZ H2471	*Tantilla gracilis*	USA	LA	Natchitoches	31.4886	–93.0291
LSUMZ H2883	*Tantilla gracilis*	USA	LA	Natchitoches	31.4886	–93.0291
LSUMZ H3352	*Tantilla gracilis*	USA	LA	Natchitoches	31.4886	–93.0291
LSUMZ H8218	*Tantilla gracilis*	USA	OK	Payne	36.1119	–97.0512
LSUMZ H8670	*Tantilla gracilis*	USA	OK	McCurtain	33.8956	–94.9159
LSUMZ H8705	*Tantilla gracilis*	USA	LA	Natchitoches	31.5395	–93.1312
LSUMZ H9298	*Tantilla gracilis*	USA	TX	Real	29.7243	–99.7823
TNHC55649	*Tantilla gracilis*	USA	TX	Travis	30.2578	–97.7447
TNHC64029	*Tantilla gracilis*	USA	TX	Bastrop	30.0842	–97.1738
TNHC84724	*Tantilla gracilis*	USA	TX	San Saba	31.0367	–98.4662
TNHCXXXX	*Tantilla gracilis*	USA	TX	Travis	30.2578	–97.7447
LSUMZ H1993	*Tantilla coronata*	USA	MS	Jackson	30.8519	–89.1840
LSUMZ H3295	*Tantilla coronata*	USA	MS	Stone	30.4293	–88.3927

Table 1: Contiued

Tissue ID	Species	Country	State	County	GPS N	GPS W
JAC 29265	*Tantilla wilcoxi*	MX	CH	NA	26.7044	–106.1555
JAC 29266	*Tantilla wilcoxi*	MX	CH	NA	26.7044	–106.1555
CLC389	*Tantilla planiceps*	MX	BS	NA	23.8084	–110.0979
CLC178	*Tantilla nigriceps*	USA	TX	Crockett	31.0236	–101.0011
CLC796	*Tantilla nigriceps*	USA	OK	Blaine	35.7504	–98.3952
MVZ 164969	*Tantilla relicta*	USA	FL	–	–	–
MZFC 11509	*Conopsis biserialis*	MX	–	–	–	–
CAS 212760	*Coluber constrictor*	USA	CA	Lake	–	–

Cytochrome *b* sequence for *Tantilla relicta* are from Holm (2008). Sequence alignments are archived on the Dryad database (doi:10.5061/dryad.8c94g44).

(mtDNA) and nuclear loci. We also created networks using median-joining and minimum-spanning methods (Bandelt *et al.*, 1999), all of which yielded identical genetic networks. For mtDNA, we constructed separate networks for the three clades that contained the greatest number of individual specimens.

Testing for the signature of range expansion across mtDNA clades

Among subtropical taxa in North America, latitudinal and longitudinal range expansions following Quaternary warming should be common. To test for the signature of spatial expansion in *T. gracilis*, we developed a non-tree-based analysis using the expectation that variation in non-recombining loci (such as mtDNA) should decrease with increasing distance from the ancestral population (Ramachandran *et al.*, 2005; Underhill & Kivisilid, 2007). This phenomenon, related to the serial founder effect, has strong theoretical underpinnings (Slatkin & Excoffier, 2012) and is supported by empirical evidence from multiple organisms, including humans (Henn *et al.*, 2012). If ongoing latitudinal and/or longitudinal post-Quaternary range expansions occurred in *T. gracilis*, we should therefore observe a significant correlation between genetic variation and the directional axis of expansion. To conduct this test, we separated latitude and longitude (decimal degrees format) of each sample into single degree bands (Fig. 3). For latitude only, there were fewer than five individual samples in three bands; therefore, we combined those bands into the next highest latitudinal band and coded that band as the median latitude. For example, because there was only a single individual found within the 33° band, this individual was combined with the individuals found in the 34° band, and the value for that band was assigned as 33.5°. We calculated within-band mean p-distance for each latitudinal or longitudinal band using MEGA v.5.2 (Tamura *et al.*, 2011). We then used linear regression to test for a relationship between genetic diversity (within-band mean p-distance) and either latitude or longitude.

Testing for the signature of demographic expansion within mtDNA clades

We used mismatch distributions to examine mtDNA of several clades of *T. gracilis* for molecular signatures associated with population growth. We generated mismatch distributions using the R package pegas (Paradis, 2010), separately for three mitochondrial clades (Northeast, Southwest and South Texas). We used the MMD function to draw histograms and estimate empirical density curves (from the observed *Tantilla* data) and curves under a stable population growth model (Rogers & Harpending, 1992). We also used pegas to calculate the following statistics for the three clades: number of haplotypes; nucleotide diversity; Ramos-Onsins and Rozas R^2 (Ramos-Onsins & Rozas, 2002); and Tajima's D (Tajima, 1989).

RESULTS

Phylogenetic relationships inferred from mitochondrial loci

We recovered very similar topologies using maximum parsimony, likelihood and Bayesian inference, so we present our results using the most likely tree from maximum likelihood analysis, with nodal support as bootstrap proportions from likelihood and parsimony analyses in addition to Bayesian posterior probabilities. We found that our sample of *Tantilla* was monophyletic, with *T. nigriceps* sister to all other sampled *Tantilla*. *Tantilla wilcoxi w*as sister to a clade composed of *T. relicta* and *T. coronata*, and *T. planiceps* was sister to *T. gracilis*. However, we note that branch support for relationships near the root of the tree was only moderate and often not supported by bootstrapping from the parsimony analyses (Fig. 3).

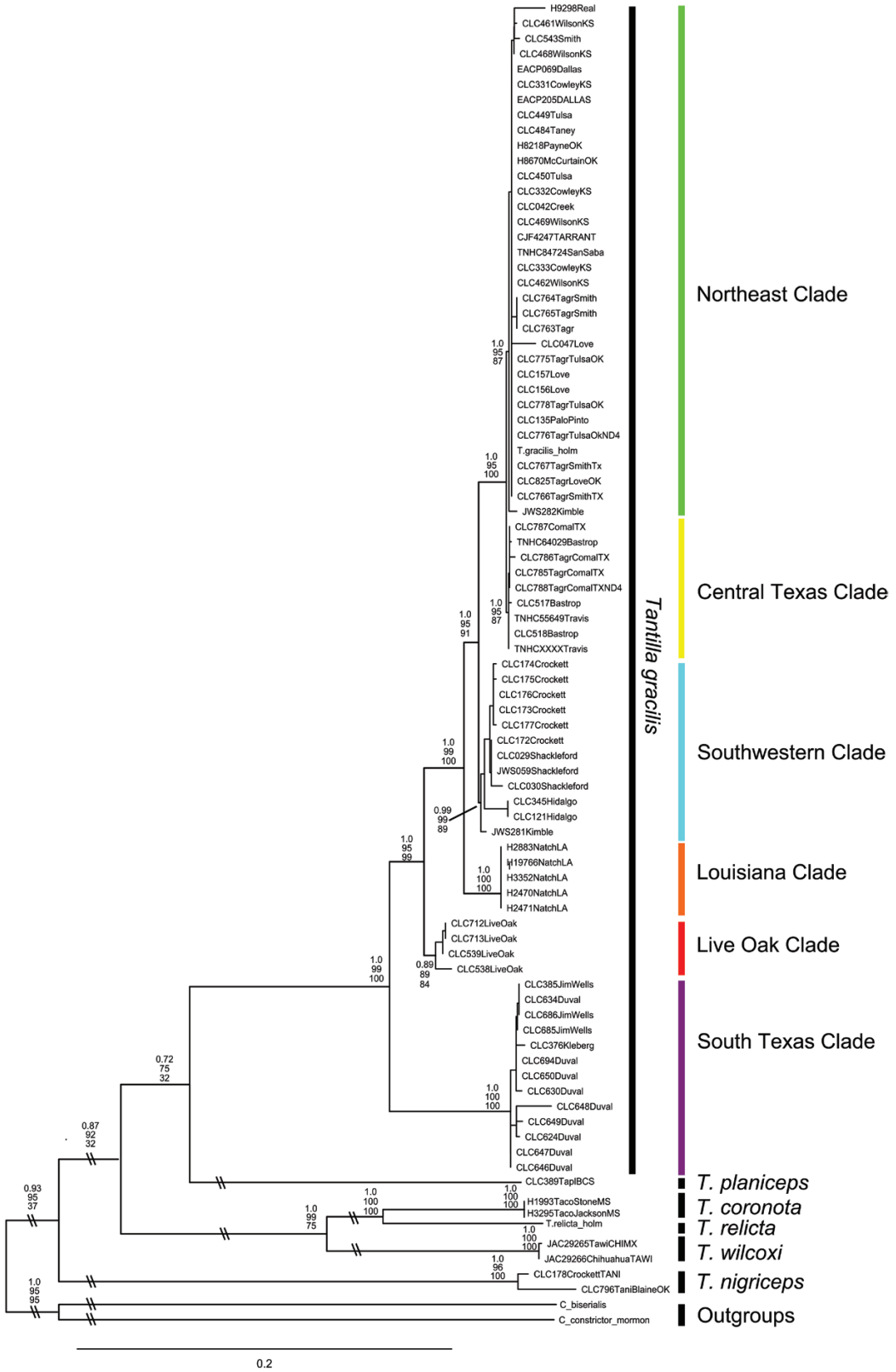

Figure 3. Phylogenetic relationships among *Tantilla gracilis* derived from mitochondrial DNA. The phylogenetic tree is the best tree from the maximum likelihood analysis. Nodal support is from Bayesian posterior probabilities (top), bootstrap support from maximum likelihood analyses (middle), and bootstrap support from maximum parsimony analyses (bottom). All three methods yielded similar topologies and nodal support.

Within *T. gracilis* (which was strongly supported as monophyletic), we found several well-supported, geographically distinctive clades (Fig. 3). The clade from south Texas, including samples from Jim Wells, Duval and Kleberg Counties (hereafter referred to as the South Texas clade), was sister to a monophyletic group consisting of all others. Interestingly, the South Texas clade did not include individuals from near the Rio Grande in extreme south Texas. Samples from Live Oak County in Texas (south of San Antonio; hereafter, the Live Oak clade) were sister to all other *T. gracilis* (exclusive of the South Texas clade), and samples from Natchitoches Parish in Louisiana (Louisiana clade) were sister to all remaining *T. gracilis*. The geographically widespread Southwestern clade included specimens from extreme south Texas (Hidalgo County), western central Texas (Kimble and Crockett Counties) and north-central Texas (Shackelford County). A clade of individuals (hereafter, the Central Texas clade) from east-central Texas (Bastrop, Comal and Travis Counties) was sister to the geographically widespread Northeast clade, which included snakes from northern and eastern Texas northward through Oklahoma, Kansas and Missouri (Fig. 3). Uncorrected divergence levels between these clades ranged from 0.7 to 5.3% (Table 2). We found only a single instance of different mitochondrial clades co-occurring within the same locality, with one individual in the Northeast clade and the other in the Southwest clade found under the same rock in Kimble County, Texas.

Geographical patterns of genetic diversity in nuclear loci

Although generally low in variation, *RAG-1* had a network structure that was consistent with some of the patterns we found using mitochondrial data (Fig. 4). Each species of *Tantilla* had a unique haplotype (nuclear allele), with all other species differing from *T. gracilis* by two or more nucleotides. Within *T. gracilis*, we found a large and widespread group composed of individuals from across most of the geographical range, and three smaller groups from South Texas, Louisiana and central Texas.

BDNF was not variable within *T. gracilis*, with only one single nucleotide polymorphism differentiating an allele of *T. gracilis* and *T. wilcoxi* from that of *T. coronata* (Fig. 4).

SNCAIP revealed one large group containing most species of *Tantilla* examined (*T. gracilis*, *T. wilcoxi*, *T. coronata* and *T. nigriceps*), with a single sample of *T. coronata* and *T. gracilis* from Live Oak County, Texas separated from the large group by a single substitution (Fig. 4).

Geographical correlates of genetic diversity within and across mitochondrial clades

We recovered evidence of demographic expansion and a northern depletion of genetic diversity in flat-headed snakes. Across the geographical range, latitude showed a significant negative correlation with mtDNA variation across clades (R^2 = 0.4298, P = 0.0396; Fig. 5A). In contrast, longitude was not significantly correlated with mtDNA variation (R^2 = 0.0104, P = 0.8278; Fig. 5B).

Genetic diversity varied within different geographically delimited clades, with the Northeast clade containing a predominant haplotype, and most other haplotypes separated by only one to three changes from the predominant haplotype haplogroup (Table 3; Fig. 6A). In contrast, both the Southwest and South Texas clades had a more even distribution of differing

Table 2. Between-clade mitochondrial DNA average divergence levels (uncorrected p-distances)

	Northeast	Louisiana	Southwestern	Live Oak	South Texas	Central Texas
Northeast (N = 37)	N/A					
Southwestern (N = 12)	0.021	N/A				
South Texas (N = 12)	0.053	0.046	N/A			
Central Texas (N = 8)	0.007	0.016	0.050	N/A		
Live Oak (N = 4)	0.029	0.022	0.037	0.025	N/A	
Louisiana (N = 5)	0.024	0.018	0.047	0.020	0.023	N/A

The analysis involved 78 nucleotide sequences of concatenated *ND4* and *cytb*. All positions with < 20% site coverage were eliminated, i.e. < 80% alignment gaps, missing data and ambiguous bases were allowed at any position. There were a total of 1347 positions in the final dataset. Analyses were conducted in MEGA5 (Tamura *et al.*, 2011). N/A indicates that data was not available.

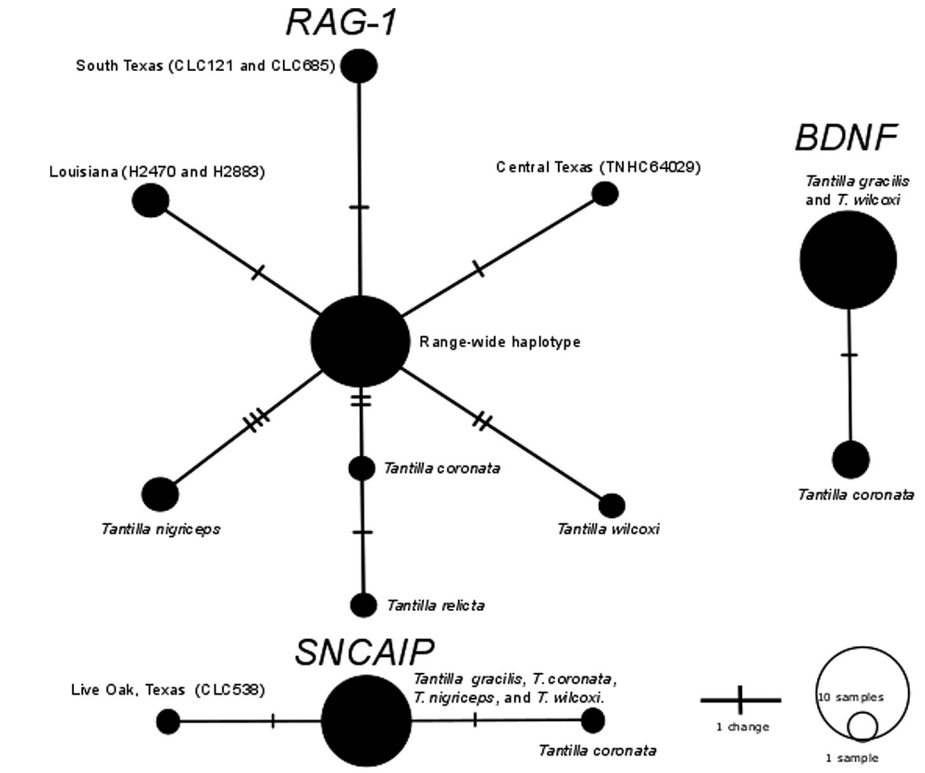

Figure 4. Genetic networks of the nuclear loci *SNCAIP*, *BDNF* and *RAG-1* from species of the genus *Tantilla*, reconstructed using the TCS parsimony method.

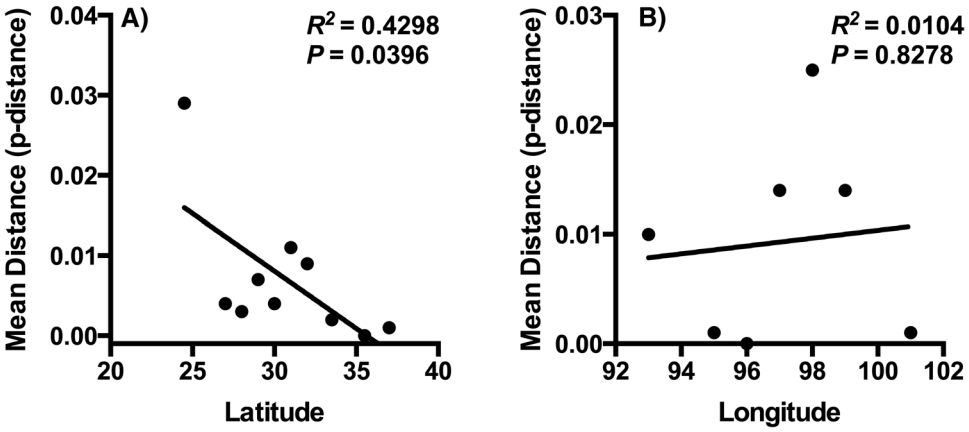

Figure 5. Relationship between mitochondrial DNA sequence variation (pairwise p-distance for *ND4* and *cytb*) and either latitude (A) or longitude (B) in *Tantilla gracilis*. Note that sequence variation declines with increasing latitude, but there is no significant relationship between longitude and sequence variation.

haplotypes and greater divergence among haplotypes (Table 3; Fig. 6B, C). In our mismatch distribution analyses, the density of pairwise distances was right skewed in all analyses, with the greatest skew observed for the Northeast clade (~80% of pairwise comparisons being zero or one total difference) compared with ~60 and 40% for the South Texas and Southwest clades, respectively (Fig. 6D–F). This distribution of pairwise differences indicates that the Northeast clade is composed of mostly similar or identical haplotypes, consistent with demographic expansion, a conclusion that is supported by the lower nucleotide diversity statistic (Table 3). In contrast, more haplotypic diversity is observed in the Southwest and South Texas clades,

Table 3. Summary statistics for three mitochondrial (mtDNA) clades of *Tantilla gracilis*

mtDNA clade	Number of haplotypes	Nucleotide diversity	R^2 test	Tajima's D
Northeast	9	0.002	0.10 ($P < 0.379$)	–3.04 ($P < 0.024$)
Southwestern	9	0.005	0.08 ($P < 0.005$)	–2.22 ($P < 0.027$)
South Texas	11	0.004	0.12 ($P < 0.174$)	–2.82 ($P < 0.005$)

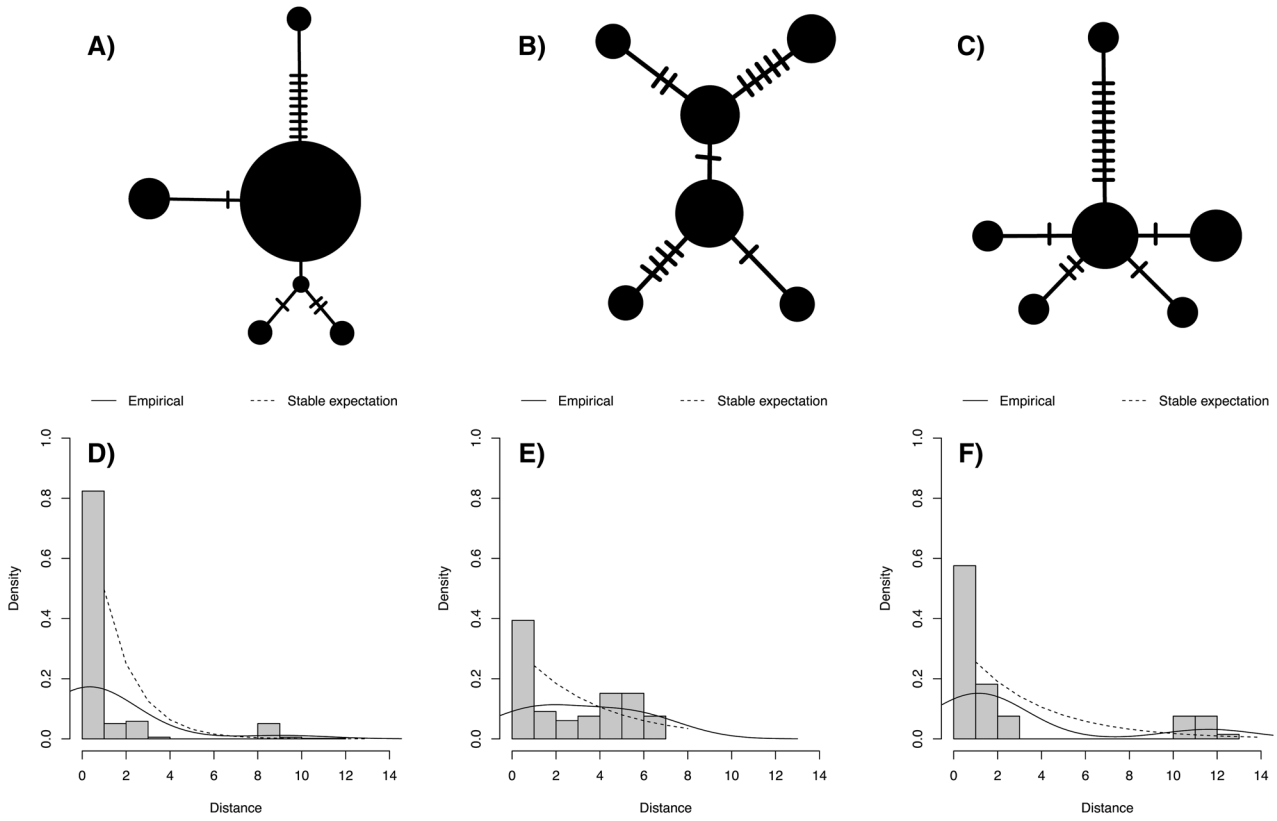

Figure 6. Haplotype networks (A–C) and mismatch distributions (D–F) for mitochondrial DNA sequences for different clades of *Tantilla gracilis*. Note that the haplotype network and mismatch distributions for the Northeast clade (A, D) show a pattern characteristic of recent population expansion, whereas the Southwest (B, E) and South Texas (C, F) clades do not.

consistent with these clades occurring in areas that harboured stable ancestral populations.

DISCUSSION

Understanding the geographical dimensions of genetic variation is important for determining how the historical demography of landscapes can influence contemporary genetic diversity within and among taxa. This is crucial because genetic diversity can shape evolutionary trajectories and the potential for adaptation. We found complex phylogenetic structure across the geographical range of *T. gracilis* when examining mitochondrial variation, an observation that was echoed weakly in some of the nuclear loci (despite minimal variation). This structure contained both geographically widespread yet genetically homogeneous clades and geographically restricted clades that contained substantial genetic variation. In the ensuing discussion, we explore our phylogenetic results and the role that Quaternary range expansion might have played in structuring contemporary genetic variation in *T. gracilis*.

PHYLOGEOGRAPHY

We found that phylogeographical relationships within *T. gracilis* were complex, with multiple geographically circumscribed clades that were limited in spatial extent (Figs 2, 4). These geographically restricted clades (Live Oak, Louisiana, Central Texas and South Texas clades) were early branching (compared with northern clades) and found in

the far south and east of the geographical range of this species. Although genetic variation in nuclear loci was minimal, we did find that the only differing genotypes were from these geographically restricted clades (Fig. 4). Together, these results suggest that these populations were isolated at some point in the past (although currently *T. gracilis* seem to be distributed continuously throughout much of their range) or that ancestral populations of *T. gracilis* occurred in the southern parts of their present distribution. Historically isolated populations are often called refugia, because they can harbour genetic diversity that is lost in other populations (or evolve new diversity). This general phylogeographical pattern of genetically distinct variants of central North American species in the eastern (e.g. Louisiana) and southern (Texas) extents of their range has been observed previously in snakes (Burbrink, 2002; Fontanella *et al.*, 2008; Guiher & Burbrink, 2008) and frogs (Moriarty *et al.*, 2008).

Our results also suggest that the Balcones Escarpment and the Edwards Plateau might have served as important suture zones between mitochondrial lineages (Fig. 2). In this study, the Balcones escarpment separates most of the clades found north (Northeast) and south of this barrier (Live Oak and South Texas), although one clade spans this barrier (Northwestern clade). The Balcones Escarpment has been found to delimit mitochondrial clades in snakes (Burbrink, 2002; Castoe *et al.*, 2007), lizards (Moseley *et al.*, 2015) and mammals (Riddle, 1995; Andersen & Light, 2012), but not frogs (Mulcahy & Mendelson, 2000; Lemmon *et al.*, 2007; Streicher *et al.*, 2012). Interestingly, the Balcones Escarpment does not represent an abrupt physical barrier to dispersal, and habitat transitions above and below the escarpment are relatively subtle. Above the Balcones escarpment is a dry and xeric habitat (the Edwards Plateau, Trans-Pecos desert and the mixed grass prairie of the Great Plains) and below is a more humid, mesic habitat (Tamaulipan thornscrub and coastal habitats; Smith & Buechner, 1947; Lieb, 1985). As such, this region may act as a filter barrier for adjacent populations or contain areas of long-term population persistence and diversification (e.g. Lost Maples).

Phylogenetic patterns in *Tantilla*

Our results have implications for understanding the phylogenetic relationships within the snake genus *Tantilla*. This is the second largest snake genus in the Western Hemisphere, with > 70 species currently described (Wilson & Mata-Silva, 2015). Our dataset has only a small sample of that diversity, but we do have representatives of all but a single species of *Tantilla* found in eastern North America (*T. oolitica*, which is likely to be closely related to *T. relicta)*. Of the species in our dataset, three are from the eastern USA or Midwest (*T. gracilis*, *T. relicta* and *T. coronata*), whereas the other two are from western North America (*T. wilcoxi* and *T. planiceps*).

Interestingly, phylogenetic relationships seem to reflect head coloration rather than geographical distribution. *Tantilla planiceps* and *T. gracilis* are sister taxa and have lighter caps that are concave in their posterior margins (Fig. 3). The clade composed of *T. wilcoxi*, *T. coronata* and *T. relicta* includes the three species with a black cap on the head and both a light and dark nuchal band (Fig. 3). *Tantilla nigriceps* is the sister taxon to all other *Tantilla* species in our dataset and has a simple convex black cap (Fig. 4). Although additional study is required to test whether this pattern persists with greater taxonomic sampling, it raises the intriguing possibility that cap morphology, which is a prominent character of this genus, might have phylogenetic signal.

Range expansion and historical demography

Using spatially explicit analyses, we found evidence that genetic variation across populations of *T. gracilis* is consistent with northern range expansion across the steppes and flatlands of central North America. Genetic diversity and the number of clades was highest in the south, whereas the northern extent of the range had a single widespread clade, with minimal genetic variation in both mitochondrial and nuclear loci. This qualitative pattern was reflected in a north-to-south gradient of mitochondrial genetic diversity, with much greater genetic diversity in the southern extent of range (Fig. 5A). Interestingly, this pattern is mostly supported by variation across clades and not within clades, suggesting that northern range expansion has been ongoing during the evolution of *T. gracilis*. Collectively, these analyses suggest a general pattern of northern range expansion, with ancestral mitochondrial haplotypes occurring in the southern and southwestern part of the range. This scenario is consistent with our finding that the southwestern extent of the range contains substantial genetic variation within geographically restricted clades. Although the lack of fossils precludes a detailed time tree of this range expansion, if we assume that *Tantilla* have a roughly similar rate of mtDNA evolution to that proposed for many other reptiles/vertebrates (1–2%/Myr; see Thorpe *et al.*, 2005), *T. gracilis* would have originated between ~2 and 6 Mya. Given that the largest between-clade divergence observed among members of the northernmost clade (Louisiana + Southwest + Central Texas + Northeast clade; Fig. 4) was 2.4% (Table 2), it follows that northern expansions are likely to have occurred within the last 1.2–2.4 Myr, during

the Pleistocene. The limited or lack of variation in populations found north of Texas is likely to indicate colonization after the Quaternary Ice Age.

Clades of flat-headed snakes varied substantially in the extent of their geographical distribution and levels of genetic diversity, potentially owing to the ecology and geography of central North America. Most of the clades are found in only a small geographical area (e.g. Louisiana, South Texas, Live Oak and Central Texas clades), but two are found throughout much of the central and southern plains of North America (Southwest and Northeast clades), and only a single clade (Northeast clade) showed the molecular signature of demographic expansion (Figs 5, 6). However, we note that R^2 and Tajima's D tests for the Northeast, Southwest and South Texas clades were all indicative of population expansion (although significant in only two cases; see Table 3). Genetic diversity varied widely among clades, with the most geographically widespread clade (Northeast clade) having modest genetic diversity and a geographically limited clade (Central Texas clade) having the greatest genetic diversity. Clades with the most restricted geographical distributions are separated from the rest of the geographical range by potential geographical or ecological barriers (e.g. the Balcones Escarpment separates the Live Oak and South Texas clades from the rest of the range) or are limited to patches of suitable habitat (e.g. the Louisiana clade). Beyond simply separating clades, these ecological and geographical factors might have limited the potential for population growth and dispersal, shaping the present geographical pattern of genetic diversity.

The range and demographic expansion patterns we observed in *T. gracilis* largely conform to the predominant phylogeographical pattern observed for vertebrates in central North America (Burbrink *et al.*, 2016), including other snakes (Cox & Chippindale, 2014; McKelvy & Burbrink, 2017), lizards (Moseley *et al.*, 2015), amphibians (Lemmon *et al.*, 2007; Fontenot *et al.*, 2011; Streicher *et al.*, 2012), birds (Ball *et al.*, 1988; Bouzat *et al.*, 1998; Johnson, 2008) and mammals (Riddle & Honeycutt, 1990; Demastes *et al.*, 2002). This shared pattern among diverse taxonomic groups suggests that many terrestrial taxa in this region have recently expanded northwards across the Great Plains, and many have a resulting legacy of depleted genetic variation in the northern segments of their distributions.

Population demography has the potential to shape the evolutionary trajectory of species. Genetic diversity harboured where populations have long persisted can potentially facilitate adaptation to altered environmental conditions. In contrast, the reduced genetic variation at the front of a spatial expansion may limit the potential for adaptation. We found that only a subset of mitochondrial and nuclear variation in *T. gracilis* is present across the large northern swathe of their geographical range, and this is a pattern that has been found in many other taxa (e.g. Fontenot *et al.*, 2011; Streicher *et al.*, 2012; Moseley *et al.*, 2015). The historical contingencies of range expansion and contraction have perhaps increased the potential of southern populations for adaptation to natural and human-mediated alterations in climate and habitat in the terrestrial fauna of central North America.

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SHARED DATA

DNA sequence alignments available on the Dryad Database (Cox *et al.*, 2018).