



Ephemeral Pleistocene woodlands connect the dots for highland rattlesnakes of the *Crotalus intermedius* group

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ABSTRACT

Aim To test how Pleistocene climatic changes affected diversification of the *Crotalus intermedius* species complex.

Location Highlands of Mexico and the south-western United States (Arizona).

Methods We synthesize the matrilineal genealogy based on 2406 base pairs of mitochondrial DNA sequences, fossil-calibrated molecular dating, reconstruction of ancestral geographic ranges, and climate-based modelling of species distributions to evaluate the history of female dispersion.

Results The presently fragmented distribution of the *C. intermedius* group is the result of both Neogene vicariance and Pleistocene pine–oak habitat fragmentation. Most lineages appear to have a Quaternary origin. The Sierra Madre del Sur and northern Sierra Madre Oriental are likely to have been colonized during this time. Species distribution models for the Last Glacial Maximum predict expansions of suitable habitat for taxa in the southern Sierra Madre Occidental and northern Sierra Madre Oriental.

Main conclusions Lineage diversification in the *C. intermedius* group is a consequence of Pleistocene climate cycling. Distribution models for two sister taxa in the northern and southern Sierra Madre Occidental and northern Sierra Madre Oriental during the Last Glacial Maximum provide evidence for the expansion of pine–oak habitat across the Central Mexican Plateau. Downward displacement and subsequent expansions of highland vegetation across Mexico during cooler glacial cycles may have allowed dispersal between highlands, which resulted in contact between previously isolated taxa and the colonization of new habitats.

Keywords

Ancestral area reconstruction, *Crotalus intermedius*, divergence dating, Mexico, niche modelling, phylogeography, Quaternary, rattlesnakes.

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INTRODUCTION

The Mexican highlands, which encompass a complex assemblage of montane biotas characterized by high endemism and diversity (Ramamoorthy *et al.*, 1993; Campbell, 1999), are one of Earth's biodiversity hotspots (Mittermeier *et al.*, 2005). They consist of four major mountain ranges predominantly covered by mixed pine–oak (*Pinus–Quercus*) woodlands, namely the north–south-trending Sierra Madre Occidental (SMOc) and Sierra Madre Oriental (SMOr) of northern Mexico, and the east–west-trending Transvolcanic Belt (TVB) and Sierra Madre

del Sur (SMS) in central and southern Mexico. The highland biotas on each range are isolated by intervening dry lowlands. Most notably, the highlands of the northern Sierra Madres are separated by hundreds of kilometres of xeric habitat formed by the Chihuahuan Desert and Central Mexican Plateau.

Although few data detail climatic shifts in Mexico during the Pleistocene (see Metcalfe *et al.*, 2000), available evidence suggests that conditions across northern and central Mexico were wetter and cooler during the Last Glacial Maximum (LGM) than they are now. McDonald (1993) reports a downward displacement of montane vegetation of at least

1000 m during glacial times. This displacement may have united highland biotas via pine–oak corridors across the Central Mexican Plateau (Baker, 1956; Martin, 1958; Duellman, 1965; Morafka, 1977; Schmidly, 1977; McCranie & Wilson, 1987; Fa & Morales, 1993; McDonald, 1993; Marshall & Liebherr, 2000). During post-glacial episodes throughout the Pleistocene, montane woodlands seem to have retracted to the highlands, thus isolating the associated biota (Metcalfe *et al.*, 2000; Anducho-Reyes *et al.*, 2008).

The highland biotas of the northern Sierra Madres appear to have had a recent connection (Marshall & Liebherr, 2000). Several dispersal corridors across central Mexico are postulated for the Pleistocene (Duellman, 1965; McCranie & Wilson, 1987; Fa & Morales, 1993; McDonald, 1993) or Pliocene (Morafka, 1977). The best evidence for these corridors comes from multiple species with closely related, disjunct, highland populations. These species are either absent or replaced by sister taxa in the Transvolcanic Belt. Martin (1958) notes numerous taxa that fit this pattern, including pines, snails, non-avian reptiles and mammals. However, confirmation of these patterns using molecular data is largely lacking (see Appendix S1 in Supporting Information). Only two studies, focused on alligator lizards (Zaldivar-Riverón *et al.*, 2005) and Mexican jays (McCormack *et al.*, 2008, 2010), estimate dates of divergence between isolated sister taxa in the northern Sierra Madres, and both pre-date Pleistocene cycles. Thus, despite being over 50 years old, the hypothesis of a Pleistocene woodland corridor across central Mexico remains largely untested.

In this study, we examine the historical diversification of the *Crotalus intermedius* species complex, a group of rattlesnakes endemic to the Mexican highlands. This group is closely associated with mixed pine–oak forests above 1900 m elevation, and it is distributed throughout all of the major mountains of Mexico (Campbell & Lamar, 2004). *Crotalus intermedius* contains three subspecies (Campbell & Lamar, 2004) disjunctly distributed primarily in pine–oak forest above 2000 m along the southern SMOr (*C. intermedius intermedius*) and SMS (*C. intermedius gloydi* and *C. intermedius omitemanus*). Similarly, *Crotalus pricei pricei* is isolated above 1900 m in the SMOc, and *Crotalus pricei miquihuanus* occurs in the SMOr (Campbell & Lamar, 2004). Finally, the sister species *Crotalus transversus* and *C. tancitarensis* are distributed in pine–oak and pine–fir (*Pinus–Abies*) forests at elevations above 2900 m on the TVB (Alvarado-Díaz & Campbell, 2004; Campbell & Lamar, 2004). These patterns offer an opportunity to test hypotheses on the role of Pleistocene pine–oak fragmentation in shaping patterns of genetic variation across the highlands of Mexico.

Recent methodological advances provide useful tools in the reconstruction of the evolutionary history of species. Mitochondrial DNA (mtDNA) sequences can be used for fossil-calibrated molecular dating. These dates can be combined with the reconstruction of ancestral geographic ranges, dispersal routes of female rattlesnakes, and climate-based modelling of species distributions to reveal insights into the factors that drive the diversification of species. Certainly, the mtDNA-only

approach is controversial (e.g. Edwards & Bensch, 2009), yet these data can detect recent geographic patterns (Moore, 1995; Hudson & Coyne, 2002; Zink & Barrowclough, 2008; Barrowclough & Zink, 2009) and lead to significant biogeographic discoveries (e.g. Upton & Murphy, 1997; Riddle *et al.*, 2000). Maternal history remains an important tool for exploring the genetic consequences of ecological history (e.g. Wiens *et al.*, 2007; Burney & Brumfield, 2009; Pyron & Burbrink, 2009), partly because genetic recombination and gene sorting do not occur.

Here, we explore the effects of Pleistocene climatic change on the diversification of the highland rattlesnakes of the *C. intermedius* group. Montane Pleistocene corridors may have facilitated contact of these rattlesnakes through dispersal. We model the distribution of *C. pricei* because this taxon currently shows the greatest extent of geographic isolation of any species in the *C. intermedius* group. If populations were connected during the last glaciation, then distribution models projected onto Late Pleistocene climates should demonstrate habitat connectivity.

MATERIALS AND METHODS

Taxon sampling and laboratory methods

Between 1999 and 2009 we collected 60 samples that represented all taxa in the *C. intermedius* group from throughout their distributions (Fig. 1; Appendix S2). Sampling was concentrated along the northern Sierra Madres to maximize geographic coverage of *C. pricei pricei* and *C. pricei miquihuanus*. Based on recent phylogenetic analyses (Murphy *et al.*, 2002; Castoe & Parkinson, 2006; Wüster *et al.*, 2008), we used *Sistrurus catenatus* and *S. miliaris* as outgroup taxa. All handling of animals followed animal use protocols approved by the University of Nevada at Las Vegas Animal Care Committee (R701-1105-203).

We sequenced three regions of the mitochondrial genome, namely a portion of the 12S and 16S ribosomal RNA genes, NADH dehydrogenase subunit 4 (ND4) and its flanking tRNAs, and the complete ATPase subunits 8 and 6 (ATPase 8, ATPase 6), following methods specified in Bryson *et al.* (2011a). These gene regions have been shown to be informative at inter- and intraspecific levels within rattlesnakes (Murphy *et al.*, 2002; Wüster *et al.*, 2005; Bryson *et al.*, 2011a). Forward and reverse sequences for each individual were edited and manually aligned using BioEDIT 5.0.9 (Hall, 1999). Identical sequences for samples from the same locality were collapsed into one haplotype. Average uncorrected pairwise divergences between these groups (*p*-distances) were determined using MEGA 4 (Kumar *et al.*, 2008).

Phylogenetic analyses

Bayesian inference (BI) trees were constructed with MRBAYES 3.1 (Ronquist & Huelsenbeck, 2003) from the combined mtDNA dataset while implementing separate models for each

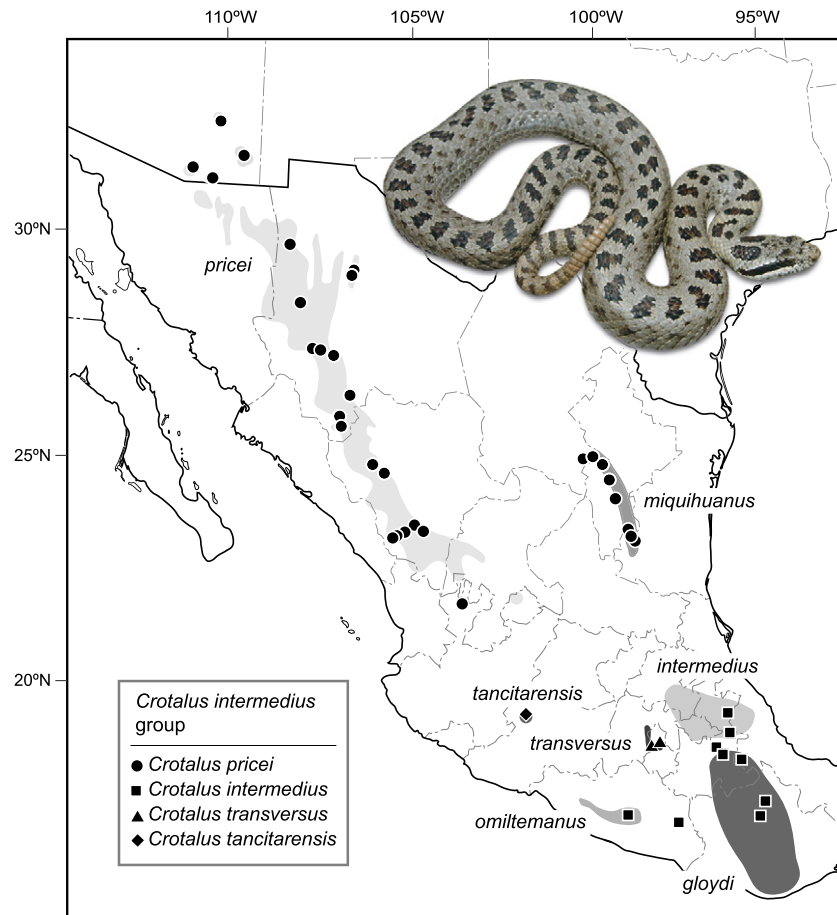


Figure 1 A map of North America depicting the sample localities and distribution (adapted from Campbell & Lamar, 2004) for the *Crotalus intermedius* species group.

gene region (ATPase 8, ATPase 6, ND4, combined tRNAs, 12S and 16S). MRMODELTEST 2.1 (Nylander, 2004) was used to select a best-fit model of evolution based on Akaike information criterion values for each partition. BI settings included random starting trees, a variable rate prior, a mean branch length exponential prior of 100, and a heating temperature of 0.03. Analyses consisted of four runs ($n_{\text{runs}} = 4$), each conducted with three heated and one cold Markov chain while sampling every 100 generations for four million generations. Output parameters were visualized using TRACER 1.5 (Rambaut & Drummond, 2007) to ascertain stationarity and whether the duplicated runs had converged on the same mean likelihood or not. We also assessed convergence using AWTY (Wilgenbusch *et al.*, 2004). Because chain convergence occurred during the first 500,000 generations of each run, we conservatively discarded all samples obtained during the first one million generations (25%) as burn-in. A 50% majority-rule consensus phylogram was estimated from a combination of the four runs. Those nodes with $\geq 95\%$ Bayesian posterior probability were considered to be strongly supported (Felsenstein, 2004).

Divergence dating

Divergence dates were estimated using a Bayesian relaxed molecular clock implemented in BEAST 1.5.4 (Drummond & Rambaut, 2007). Dates were inferred for a reduced dataset,

which included one individual from each geographic group for each taxon. Sequences from several North American pitvipers were used to calibrate the tree (Appendix S2). Best-fit models of evolution were re-estimated from the new dataset using MRMODELTEST. We implemented an uncorrelated lognormal clock and node constraints obtained from the fossil and geological record with lognormal distributions. Analyses were run for 40 million generations, with samples retained every 1000 generations and a Yule tree prior. TRACER was used to confirm acceptable mixing and likelihood stationarity of the MCMC chain, burn-in, and adequate effective sample sizes (> 200 for each estimated parameter). Two independent analyses were run on an unpartitioned dataset because a partitioned-by-gene dataset resulted in effective sample sizes below 50 for several parameters. After discarding the first four million generations (10%) as burn-in, we combined the trees and parameter estimates from the two runs using LOGCOMBINER 1.4.8 (Drummond & Rambaut, 2007). We summarized parameter values on the maximum clade credibility tree using TREEANNOTATOR 1.4.8 (Drummond & Rambaut, 2007) with the posterior probability limit set to 0.5 and summarizing mean node heights.

Calibration was based on North American pitvipers using the same approach as Bryson *et al.* (2011a), as follows.

1. The stem of *Sistrurus* was constrained with a zero offset (hard upper bound) of 8 million years ago (Ma), a lognormal mean of 0.01, and a lognormal standard deviation of 0.76. This

produced a median age centred at 9 Ma and a 95% prior credible interval extending to the beginning of the Clarendonian, 11.5 Ma (Holman, 2000).

2. The node representing the most recent common ancestor of *Agkistrodon contortrix* was given a zero offset of 6 Ma, a lognormal mean of 0.01, and a lognormal standard deviation of 0.42, producing a median age of 7 Ma and a 95% prior credible interval extending to the start of the Late Hemphillian, 8 Ma (Holman, 2000).

3. The node representing the most recent common ancestor of *Crotalus ruber* plus *C. atrox* was given a lognormal mean of 1.1 and a lognormal standard deviation of 0.37, resulting in a median age centred at the climax of the formation of the Sea of Cortés and the development of the Bouse embayment 3 Ma, and a 95% prior credible interval extending to the beginning of the development of the Sea of Cortés, 5.5 Ma (Carreño & Helenes, 2002, and references therein). No zero offset was used.

Ancestral area reconstruction

Ancestral areas for *C. pricei* were reconstructed using a stochastic model of geographic range evolution (dispersal–extinction–cladogenesis, DEC) implemented in LAGRANGE 2.0.1 (Ree & Smith, 2008). The maximum clade-credibility tree from BEAST was pruned of outgroups and used in analyses, and each representative taxon in the genealogy was assigned to one of six biogeographic areas based on distributions (Figs 1 & 2) as follows: northern SMOc, southern SMOc, northern SMOr, southern SMOr, TVB and SMS. The region near the eastern TVB in Puebla, Veracruz, and northern Oaxaca (Fig. 3) has a complex geological history; it contains geological and biotic elements of the SMOr, TVB and SMS (Marshall & Liebherr, 2000; Salinas-Moreno *et al.*, 2004; Corona *et al.*, 2007; Paniagua & Morrone, 2009). For our analyses, we considered it as part of the SMOr, based on faunal rather than geological affinities (Paniagua & Morrone, 2009). All range sizes were constrained to be composed of at most two regions, and the root age of the tree was that estimated in the BEAST analyses. We constrained widespread ancestors to spatially adjacent areas to exclude unrealistic ranges (e.g. northern SMOc + SMS).

Species distribution modelling

We used MAXENT 3.3.2 (Phillips *et al.*, 2006) to reconstruct the current and LGM climatic niches of *C. pricei pricei* and *C. pricei miquihuanus*, because this outperformed other modelling approaches (Elith *et al.*, 2006). We used 59 geo-referenced localities to construct the models (Appendix S3) based on data from HerpNet (<http://herpnet.org>), published records with GPS coordinates (Lemos-Espinal & Smith, 2007a,b), GPS coordinates supplied by the collectors of our genetic samples, and by plotting museum records with detailed localities on Google Earth (<http://earth.google.com>). Records from HerpNet with geographic uncertainty greater than 5 km were

excluded. Detailed electronic state maps (<http://dgp.sct.gob.mx/index.php?id=440>) were used to help locate place names in Mexico. Occurrences were assigned to one of three biogeographic regions (northern SMOc, southern SMOc and northern SMOr), and distribution models were constructed for each region.

The initial models used 19 bioclimatic layers representing current climatic trends, seasonality, and extremes of temperature and precipitation. The data were clipped to cover most of Mexico and part of the south-western United States (north-western corner 38.83°, –118.04°; south-eastern corner 15.02°, –94.25°). Initial distribution models were constructed using values extracted from the grid cells of each bioclimatic layer that contained at least one occurrence record. Two layers were considered to be highly correlated when the Pearson's correlation coefficient was greater than 0.75 (Rissler *et al.*, 2006; Rissler & Apodaca, 2007). Layers highly correlated with three or more other layers were omitted from subsequent analysis, and only one layer between highly correlated pairs was retained for subsequent MAXENT runs. The final models used the following nine bioclimatic layers: Bio1, annual mean temperature; Bio2, mean diurnal range; Bio3, isothermality; Bio6, minimum temperature of the coldest month; Bio8, mean temperature of the wettest quarter; Bio9, mean temperature of the driest quarter; Bio15, precipitation seasonality; Bio 16, precipitation of the wettest quarter; and Bio17, precipitation of the driest quarter.

We ran MAXENT using logistic output, default settings and random seeding. To test the robustness of the MAXENT models, we used cross-validation, dividing presence points into five groups and running five iterations while using a different group for each run. Thus, 20% of the presence points were used as test points and 80% were used for training (Nogués-Bravo, 2009). Model performance was evaluated using the default method of determining the area under the receiver operating characteristic curve.

The distribution models were projected onto simulated climates for the LGM (*c.* 21 ka) derived from the Community Climate System Model (Otto-Bliesner *et al.*, 2006). Habitat suitability was displayed in ARCGIS 9.2. (ESRI Inc., Redlands, CA, USA, 2007) using two thresholds: the 10th percentile training presence and lowest training presence thresholds. The former used the lowest threshold value that did not predict the most extreme 10% of the presence observations, assuming that they may have represented recording errors or atypical climatic conditions within a grid cell (Morueta-Holme *et al.*, 2010). The latter threshold only predicted grid cells at least as suitable as those where presence had been recorded (Pearson *et al.*, 2007).

RESULTS

Sequence characteristics and maternal genealogy

The dataset consisted of 2406 aligned nucleotide positions. Models of sequence evolution included HKY + G (ATPase 8,

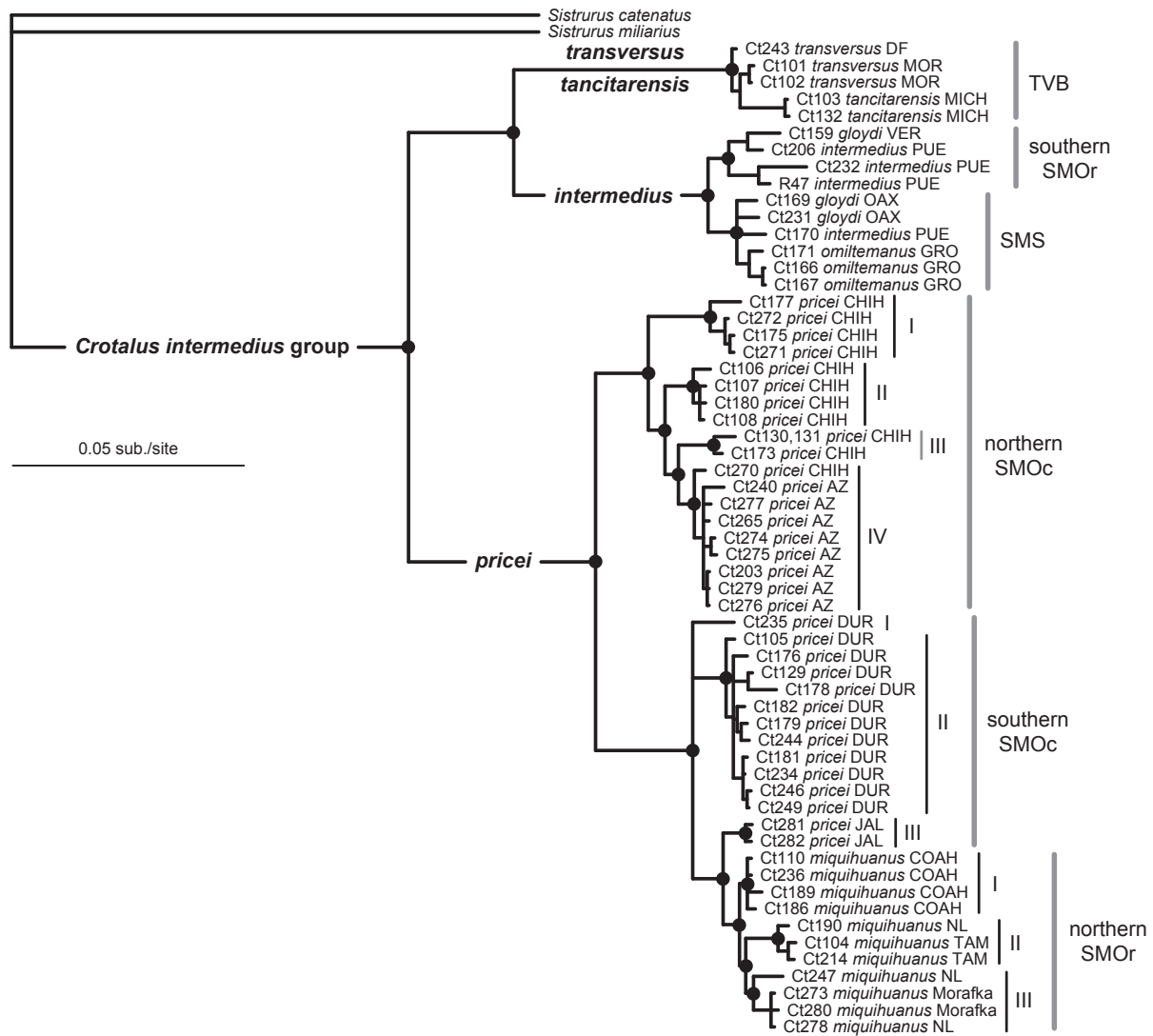


Figure 2 Maternal genealogy of the *Crotalus intermedius* species group based on mixed-model Bayesian inference of mitochondrial DNA sequence data. Major geographic groups are indicated, and abbreviations refer to the biogeographic areas delineated in Fig. 3: northern Sierra Madre Occidental (SMOc), southern SMOc, northern Sierra Madre Oriental (SMOr), southern SMOr, Transvolcanic Belt (TVB) and Sierra Madre del Sur (SMS). Roman numerals indicate additional clades nested within geographic groups. All major nodes that received $\geq 95\%$ Bayesian posterior probability support are depicted with black dots.

tRNAs), GTR + I + G (ATPase 6), GTR + G (ND4), GTR + I (12S) and HKY + I (16S). Nearly all nodes on the tree were highly supported (Fig. 2), indicating that the suite of genes used was adequate for resolving both interspecific and intraspecific relationships. All sequences were deposited in GenBank (accession numbers JN022789–JN022953).

Considerable geographic partitioning occurred within two of the three species (Fig. 2). *Crotalus pricei* had two major clades, with several geographically structured groups. The *p*-distances between the two major clades averaged 2.4%. One major clade consisted of *C. pricei pricei* from southern Chihuahua northwards to Arizona. This clade was further divided into four geographic groups. The second major clade consisted of two geographically restricted groups of *C. pricei pricei* from the SMOc south of Chihuahua, plus three groups

of *C. pricei miquihuanus* from the northern SMOr. *Crotalus intermedius* had two geographic groups, one distributed along the southern end of the SMOr and one within the SMS. The *p*-distance between groups averaged 0.6%. The subspecies were not composed of exclusive groups. Furthermore, one sample of *C. intermedius intermedius* from the SMOr near the Puebla/Oaxaca border (Ct170) nested within the SMS (Fig. 2), suggesting either dispersal or incomplete lineage sorting. *Crotalus tancitarenensis* nested within *C. transversus*.

Divergence times and ancestral areas

The GTR + I + G model was selected for the BEAST analyses. Dating estimates (Fig. 3, Table 1) suggested that diversification in the *C. intermedius* group began in the late Neogene. Further

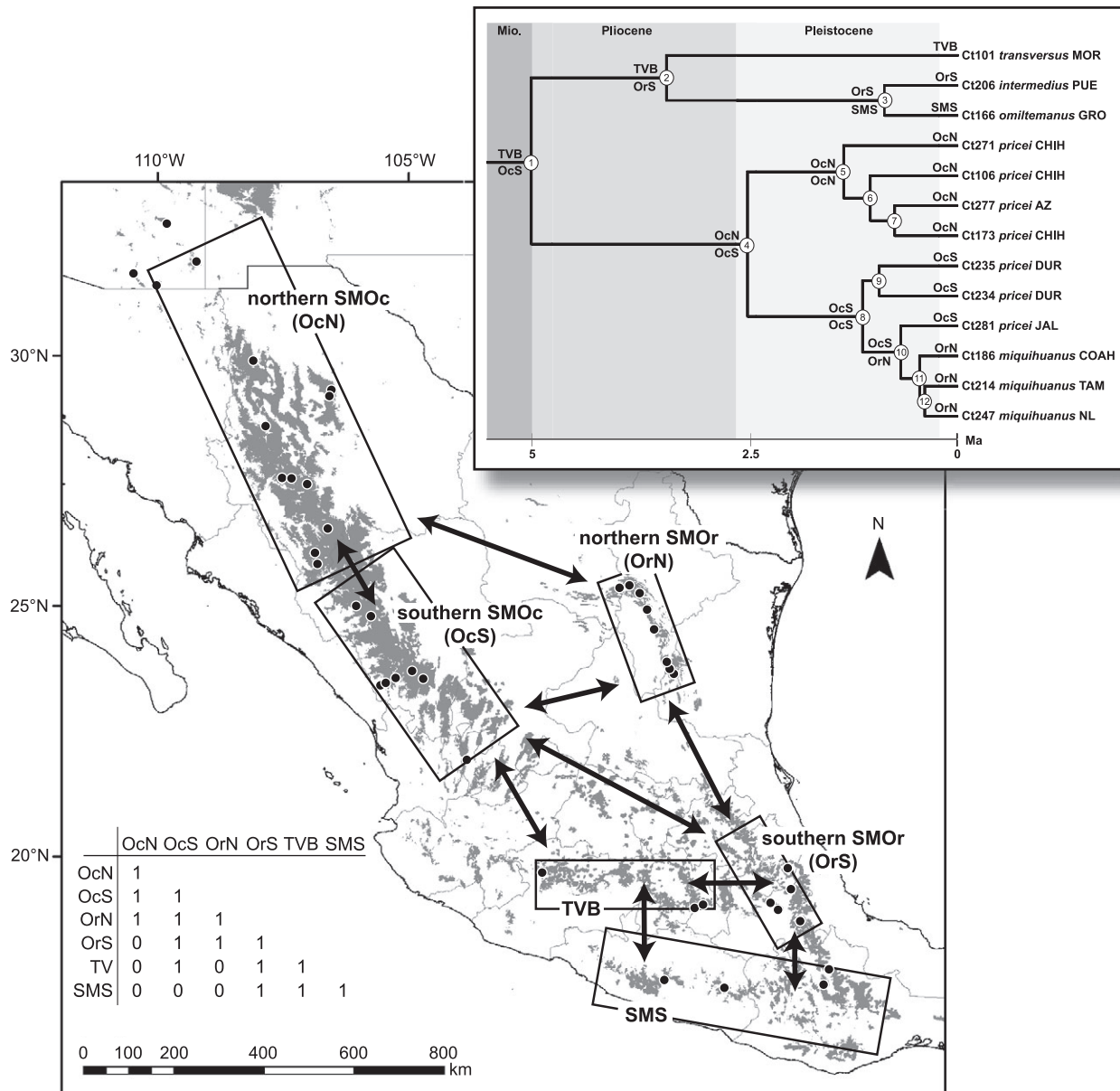


Figure 3 Biogeographic reconstruction based on dispersal–extinction–cladogenesis (DEC) modelling. Arrows show dispersal constraints among geographically adjacent areas used to create the area adjacency matrix in the lower left corner. The inset shows the chronogram inferred using Bayesian relaxed clock phylogenetic analyses for lineage divergences within the *Crotalus intermedius* species group. Optimal ancestral areas with the highest likelihood scores and the highest probabilities are presented at each node. Alternative ancestral areas within 2 log-likelihood units and estimated divergence dates for numbered nodes can be found in Table 1. Biogeographic areas are as follows: northern Sierra Madre Occidental (SMOc), southern SMOc, northern Sierra Madre Oriental (SMOr), southern SMOr, Transvolcanic Belt (TVB) and Sierra Madre del Sur (SMS). Localities of genetic samples used in study are overlaid on mixed pine–oak forests above 1900 m, shown in grey.

geographic partitioning appeared to have been generated during the Quaternary.

Optimal ancestral areas with the highest likelihood scores and probabilities were identified at each node (Fig. 3). Alternative ancestral areas within 2 log-likelihood units are summarized in Table 1. Two nodes with alternative ancestral areas (nodes 1 and 8) appeared unresolved; probabilities between alternative reconstructions differed by less than 10%. Initial diversification within the *C. intermedius* group (node 1) occurred between the southern SMOc and either the TVB or

the southern SMOr. This was followed by a probable northward dispersal into the northern SMOc from the southern SMOc (node 4), and then an eastward dispersal from the TVB to the southern SMOr (node 2). Subsequent diversification in the SMOc may have involved either *in situ* diversification in both the northern (node 5) and southern (node 8) regions, or *in situ* diversification in the southern SMOc followed by eastward dispersal into the northern SMOr (node 8). Colonization of the SMS appeared to have occurred through dispersal from the southern SMOr (node 3).

Table 1 Estimated divergence dates within the *Crotalus intermedius* species group based on Bayesian relaxed phylogenetic analyses of mitochondrial DNA sequence data. Numbers refer to nodes identified in Fig. 2. Posterior mean ages and 95% highest posterior density (HPD) intervals are provided in millions of years ago (Ma). Ancestral areas reconstructed for each node are followed by relative probability values. Area reconstruction at each node is the split of areas inherited by the two descendant branches (upper branch followed by lower branch). The optimal ancestral areas with the highest probabilities were selected among the alternatives and are presented in Fig. 3. Abbreviations are as follows: SMOc, Sierra Madre Occidental; SMOOr, Sierra Madre Oriental; TVB, Transvolcanic Belt; SMS, Sierra Madre del Sur.

Node	Posterior mean age in Ma (95% HPD)	Ancestral area (relative probability)
1	5.2 (3.7–6.9)	TVB/southern SMOc (0.34) Southern SMOOr/southern SMOc (0.26) Southern SMOOr/northern SMOOr (0.12)
2	3.5 (2.1–5.1)	TVB/southern SMOOr (0.29) Southern SMOOr/southern SMOOr (0.17) TVB/SMS (0.15) TVB/TVB (0.07) Southern SMOOr + TVB/southern SMOOr (0.06) Southern SMOc/southern SMOOr (0.06)
3	0.9 (0.4–1.6)	Southern SMOOr/SMS (0.86)
4	2.6 (1.6–3.7)	Northern SMOc/southern SMOc (0.65) Northern SMOc/northern SMOOr (0.12)
5	1.4 (0.8–2.1)	Northern SMOc/northern SMOc (0.94)
6	1.1 (0.5–1.7)	Northern SMOc/northern SMOc (0.99)
7	0.8 (0.3–1.3)	Northern SMOc/northern SMOc (1.0)
8	1.2 (0.7–1.7)	Southern SMOc/southern SMOc (0.50) Southern SMOc/southern SMOc + northern SMOOr (0.47)
9	1.0 (0.5–1.5)	Southern SMOc/southern SMOc (1.0)
10	0.7 (0.4–1.1)	Southern SMOc/northern SMOOr (0.93)
11	0.5 (0.2–0.8)	Northern SMOOr/northern SMOOr (0.99)
12	0.4 (0.2–0.7)	Northern SMOOr/northern SMOOr (1.0)

Species distribution modelling

All operating characteristic curve values were high (> 0.99) for the three biogeographic regions, indicating that the models performed better than random (Raes & ter Steege, 2007). The model for current climatic conditions in the northern SMOc (Fig. 4a) obtained a core distribution extending from the highlands of Durango to north-eastern Sonora, with a scattering of disjunct suitable habitat in the sky islands north of the SMOc. A few small disjunct patches were predicted to occur along the TVB. The LGM model (Fig. 4b) illustrated a similar core distribution, but no suitable climate in the sky island region and an increase in suitable climate in the TVB. Areas of highest suitability were mostly centred within the core distribution in the northern SMOc, which appeared to have remained geographically stable since the LGM.

Models for the current southern SMOc (Fig. 4c) depicted a large patch of suitable climate in the highlands of Durango that overlapped habitat predicted as being suitable for the northern SMOc. A few small patches of suitable area also occurred along the TVB. The LGM model for the southern SMOc (Fig. 4d) predicted a dramatic increase in areas with suitable climate. This result differed from the LGM models for the northern SMOc. The greatest increase occurred along the southern SMOc, the Central Mexican Plateau and the TVB, effectively producing a historical continuation of suitable climate between these regions. Furthermore, a large disjunct area of high suitability was predicted from the northern SMOOr throughout the adjacent high-elevation regions of central Coahuila and Nuevo León.

The model for the current distribution of *C. pricei miquihuanus* (Fig. 4e) identified regions of suitable climate in two main areas, the northern SMOOr and eastern TVB. Isolated patches of moderately suitable habitat extended westwards from the SMOOr into the northern Central Mexican Plateau. The model for the LGM (Fig. 4f) extended the amount of suitable habitat approximately 100 km to the west and 150 km to the south along the northern Central Mexican Plateau.

Taken together, the species distribution models suggested that habitat with climate suitable for *C. pricei pricei* and *C. pricei miquihuanus* was contiguous in the Central Mexican Plateau during the LGM and presumably during the many other glacial periods. The models indicated that the current distributions of the northern SMOc and southern SMOc overlap today about as much as they did during the LGM. Current climatic conditions favourable to *C. pricei pricei* (northern and southern SMOc) and *C. pricei miquihuanus* did not overlap in the models.

DISCUSSION

Pleistocene pine–oak woodland connectivity

Most of the geographic partitioning of lineages within the *C. intermedius* group appears to have been generated during the Quaternary (Fig. 3; Table 1). The majority of presently isolated populations are genetically similar (Fig. 2), and the ancestral area reconstruction (Fig. 3) suggests several dispersal events. Ephemeral Quaternary corridors across current montane barriers may help to explain the lack of deep lineage structure observed in co-distributed highland species. For example, *C. intermedius* distributed along the SMS probably recently dispersed into this region. Little discernible lineage structuring occurs across the Rio Verde basin along the border of Oaxaca and Guerrero. This basin is a well-characterized barrier to dispersal for several highland taxa, including birds (García-Moreno *et al.*, 2004; Navarro-Sigüenza *et al.*, 2008; Puebla-Olivares *et al.*, 2008), small mammals (Edwards & Bradley, 2002) and other montane rattlesnakes (Bryson *et al.*, 2011a).

Highland corridors across the Central Mexican Plateau

Interglacial periods, such as the current one, represent only about 6% of the total duration of the Pleistocene (Van

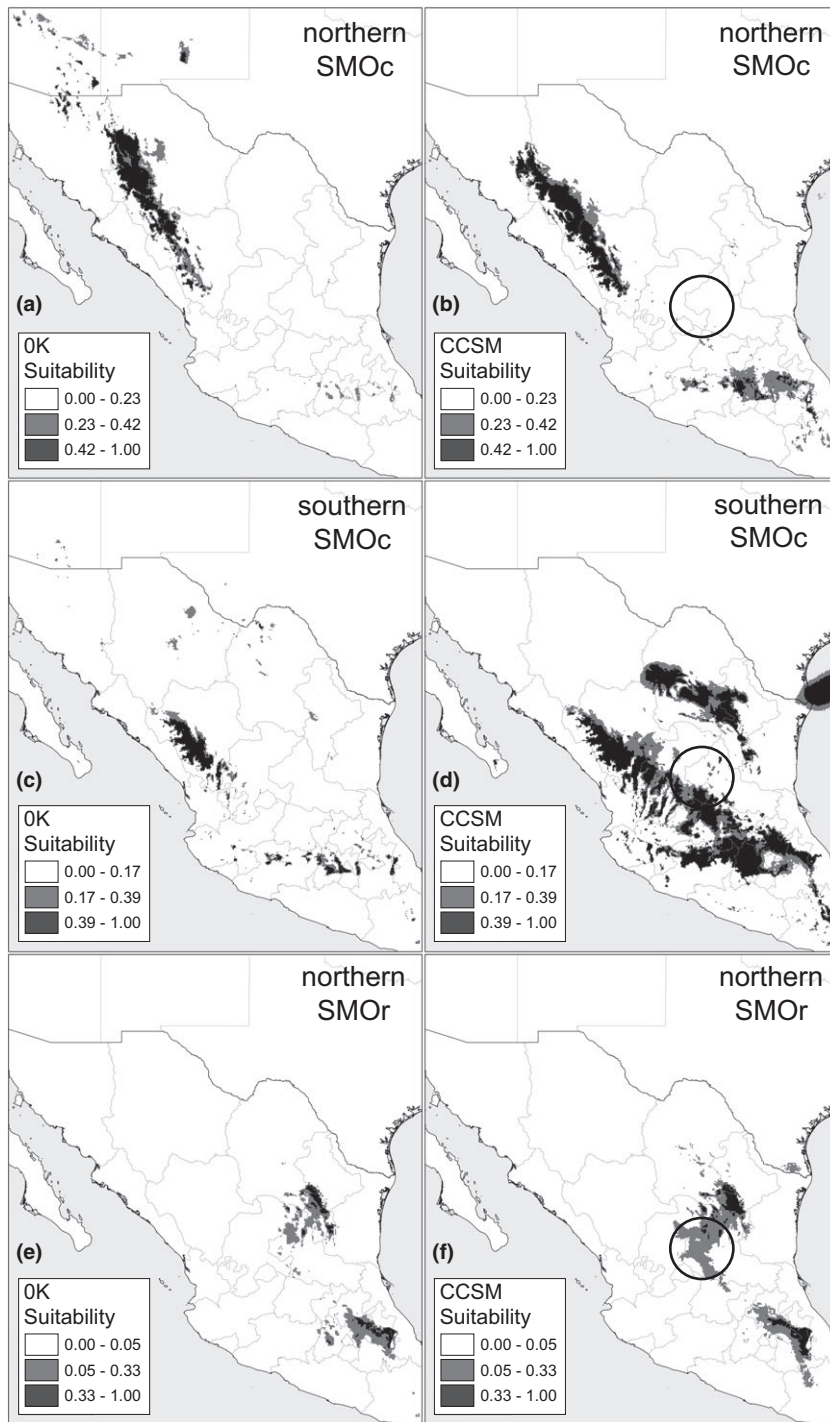


Figure 4 Species distributions models for *Crotalus pricei pricei* and *C. pricei miquihuanus* under current and Last Glacial Maximum (LGM, c. 21 ka) climate scenarios based on three biogeographic regions: northern Sierra Madre Occidental (SMOc), southern SMOc, and northern Sierra Madre Oriental (SMOr). (a) Northern SMOc, current; (b) northern SMOc, LGM; (c) southern SMOc, current; (d) southern SMOc, LGM; (e) northern SMOr, current; (f) northern SMOr, LGM. These taxa may have been connected through pine–oak woodlands across the northern Central Mexican Plateau (circled) at the LGM.

Devender & Burgess, 1985). Many of the isolated biotas of the Mexican highlands are ephemeral, and what we see today is likely to be atypical of the Quaternary. The two taxa with the greatest degree of geographic isolation, *C. pricei pricei* and *C. pricei miquihuanus*, probably diverged in the Pleistocene (Fig. 3; Table 1). Given that these two subspecies are isolated by hundreds of kilometres of xeric habitat – the Chihuahuan Desert and Central Mexican Plateau – these results are somewhat perplexing. Recent divergence is postulated for a variety of other disjunct highland taxa shared between the

SMOc and SMOr (Martin, 1958; Appendix S1), and we provide the first genetic confirmation of the pattern. Previously estimated divergence dates for imbricate alligator lizards (Zaldivar-Riverón *et al.*, 2005) and Mexican jays (McCormack *et al.*, 2008, 2010) suggest splits that pre-date the Quaternary glacial–interglacial cycles.

Several historical corridors of montane vegetation are thought to have existed across central Mexico during the LGM (Duellman, 1965; McCranie & Wilson, 1987; Fa & Morales, 1993; McDonald, 1993). Because *C. pricei* is tightly

linked to highland pine–oak woodlands (Campbell & Lamar, 2004), its distribution serves as a proxy for that of pine–oak forests. When projected onto LGM climates, the distribution models for *C. pricei* (Fig. 4) provide evidence for the expansion of pine–oak habitat across the Central Mexican Plateau. Although the distribution of *C. pricei pricei* in the northern SMOc appears little changed, with a predicted loss of habitat only in the north, our models predict a dramatic expansion of suitable habitat for *C. pricei pricei* in the southern SMOc. The models for *C. pricei miquihuanus* in the SMOr also suggest an expansion of suitable habitat across the Central Mexican Plateau.

Our analyses support a Pleistocene divergence between SMOc and SMOr for *C. pricei* as well as the existence of montane vegetation corridors across central Mexico. Ancestral area reconstruction predicts a probable dispersal of *C. pricei* from the southern SMOc (Fig. 3; Table 1), although the route into the SMOr remains uncertain. Distribution models (Fig. 4d) predict little habitat connectivity between these regions during the LGM. Therefore, it seems that *C. pricei* dispersed into the northern SMOr prior to the LGM and then reconnected with *C. pricei* in the southern SMOc through a pine–oak corridor extending across the northern Central Mexican Plateau. This scenario provides support for the alternative ancestral area reconstruction at node 8 (Table 1). The series of small isolated sierras with fragments of pine–oak forest scattered across western San Luis Potosí may be remnants of this connecting corridor.

Late Neogene diversification and the Mexican highlands

In addition to Pleistocene fragmentation of pine–oak habitats, it is likely that Neogene vicariance also affected the *C. intermedius* group. The TVB is one of the predominant geographic features of Mexico, and its uplift may be responsible for diversification in the *C. intermedius* group and other taxa (Mulcahy *et al.*, 2006; Morrone, 2009, 2010; Bryson *et al.*, 2011a). Timing of the basal split in the *C. intermedius* group, around 5 Ma (Fig. 3, Table 1), coincides with the primary, Late Miocene orogenesis of the TVB (Rosas-Elguera *et al.*, 2003). This estimation is similar to dates for other groups, including toads (4.2–7.6 Ma; Mulcahy & Mendelson, 2000), heroine cichlids (4.7–8.7 Ma; Hulsey *et al.*, 2004), Mexican jays (4.4–9.4 Ma; McCormack *et al.*, 2008), montane rattlesnakes (5–8 Ma; Bryson *et al.*, 2011a) and Middle American gophersnakes (5.0–8.6 Ma; Bryson *et al.*, 2011b). Furthermore, ancestral area reconstruction suggests that diversification in the *C. intermedius* group began after a TVB/southern SMOc split.

Rattlesnakes in the TVB (*C. transversus* and *C. tancitarensis*) appear to have diverged from *C. intermedius* sometime in the Pliocene (Fig. 3; Table 1). The Balsas Basin, an arid lowland depression between the TVB and SMS that formed in the Pliocene and Pleistocene (Becerra & Venable, 1999; Marshall & Lieberr, 2000), probably prevents southward dispersal from

the TVB. Ancestral area reconstruction suggests divergence between species in the TVB and *C. intermedius* in the southern SMOc. A similar history has been proposed for several other co-distributed highland taxa (Sullivan *et al.*, 1997; León-Paniagua *et al.*, 2007; Anducho-Reyes *et al.*, 2008; Bryson *et al.*, 2011a).

CONCLUSIONS

Our examination of the matrilineal history of the *C. intermedius* group suggests that Pleistocene climate cycling played a major role in driving lineage diversification. Taxa in this species group are most often associated with montane highlands such as pine–oak forests. Downward displacement and subsequent expansions of highland vegetation across Mexico during cooler glacial cycles, which occurred throughout most of the Pleistocene, may have allowed dispersal, and thus gene flow, between highland populations. Previously isolated rattlesnakes and other co-distributed highland species appear to have been reunited by the formation of new habitat corridors. Populations of *C. pricei* presently isolated along the northern Sierra Madres may have been in contact as recently as the LGM. This finding suggests that, despite separation by hundreds of kilometres of xeric habitat, the highland biotas of the SMOc and SMOr were recently connected. This provides the first genetic confirmation of previous hypotheses of montane Pleistocene corridors across the Central Mexican Plateau. In the age of genomics (Haussler *et al.*, 2009), maternal history remains an important tool for exploring the genetic consequences of ecological history.

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REFERENCES

- Alvarado-Díaz, J. & Campbell, J.A. (2004) A new montane rattlesnake (Viperidae) from Michoacán, Mexico. *Herpetologica*, **60**, 281–286.
- Anducho-Reyes, M.A., Cognato, A.I., Hayes, J.L. & Zuniga, G. (2008) Phylogeography of the bark beetle *Dendroctonus mexicanus* Hopkins (Coleoptera: Curculionidae: Scolytinae). *Molecular Phylogenetics and Evolution*, **49**, 930–940.
- Baker, R.H. (1956) Mammals of Coahuila, Mexico. *University of Kansas Publications, Museum of Natural History*, **9**, 127–335.
- Barrowclough, G.F. & Zink, R.M. (2009) Funds enough, and time: mtDNA, nuDNA and the discovery of divergence. *Molecular Ecology*, **18**, 2934–2936.
- Becerra, J.X. & Venable, D.L. (1999) Macroevolution of insect–plant associations: the relevance of host biogeography to host affiliation. *Proceedings of the National Academy of Sciences USA*, **96**, 12626–12631.
- Bryson, R.W., Murphy, R.W., Lathrop, A. & Lazcano-Villareal, D. (2011a) Evolutionary drivers of phylogeographical diversity in the highlands of Mexico: a case study of the *Crotalus triseriatus* species group of montane rattlesnakes. *Journal of Biogeography*, **38**, 697–710.
- Bryson, R.W., García-Vázquez, U.O. & Riddle, B.R. (2011b) Phylogeography of Middle American gophersnakes: mixed responses to biogeographical barriers across the Mexican Transition Zone. *Journal of Biogeography*, **38**, 1570–1584.
- Burney, C.W. & Brumfield, R.T. (2009) Ecology predicts levels of genetic differentiation in Neotropical birds. *The American Naturalist*, **174**, 358–368.
- Campbell, J.A. (1999) Distribution patterns of amphibians in Middle America. *Patterns of distribution of amphibians: a global perspective* (ed. by W.E. Duellman), pp. 111–210. Johns Hopkins University Press, Baltimore, MD.
- Campbell, J.A. & Lamar, W.W. (2004) *Venomous reptiles of the Western Hemisphere*. Cornell University Press, Ithaca, NY.
- Carreño, A.L. & Helenes, J. (2002) Geology and ages of the islands. *A new island biogeography of the Sea of Cortés* (ed. by T.J. Case, M.L. Cody and E. Ezcurra), pp. 14–40. Oxford University Press, New York.
- Castoe, T.A. & Parkinson, C.L. (2006) Bayesian mixed models and the phylogeny of pitvipers (Serpentes: Viperidae). *Molecular Phylogenetics and Evolution*, **39**, 91–110.
- Corona, A.M., Toledo, V.H. & Morrone, J.J. (2007) Does the Trans-Mexican Volcanic Belt represent a natural biogeographic unit? An analysis of the distributional patterns of Coleoptera. *Journal of Biogeography*, **34**, 1008–1015.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Duellman, W.E. (1965) A biogeographic account of the herpetofauna of Michoacán, México. *University of Kansas Publications, Museum of Natural History*, **15**, 1–709.
- Edwards, C.W. & Bradley, R.D. (2002) Molecular systematics and historical phylogeography of the *Neotoma mexicana* species group. *Journal of Mammalogy*, **83**, 20–30.
- Edwards, S. & Bensch, S. (2009) Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. *Molecular Ecology*, **18**, 2930–2933.
- Elith, J., Graham, C.H., Anderson, R.P. et al. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.
- Fa, J.E. & Morales, L.M. (1993) Patterns of mammalian diversity in Mexico. *Biological diversity in Mexico: origins and distribution* (ed. by T.P. Ramamoorthy, R. Bye, A. Lot and J. Fa), pp. 319–361. Oxford University Press, New York.
- Felsenstein, J. (2004) *Inferring phylogenies*. Sinauer Associates, Sunderland, MA.
- García-Moreno, J., Navarro-Sigüenza, A.G., Peterson, A.T. & Sánchez-González, L.A. (2004) Genetic variation coincides with geographic structure in the common bush-tanager (*Chlorospingus ophthalmicus*) complex from Mexico. *Molecular Phylogenetics and Evolution*, **33**, 186–196.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.
- Hausser, D., O'Brien, S.J., Ryder, O.A. et al. (2009) Genome 10K: a proposal to obtain whole-genome sequence for 10 000 vertebrate species. *Journal of Heredity*, **100**, 659–674.
- Holman, J.A. (2000) *Fossil snakes of North America: origin, evolution, distribution, paleoecology*. Indiana University Press, Indianapolis, IN.
- Hudson, R.R. & Coyne, J.A. (2002) Mathematical consequences of the genealogical species concept. *Evolution*, **56**, 1557–1565.
- Hulsey, C.D., García de León, F.J., Johnson, Y.S., Hendrickson, D.A. & Near, T.J. (2004) Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. *Molecular Phylogenetics and Evolution*, **31**, 754–764.
- Kumar, S., Dudley, J., Nei, M. & Tamura, K. (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics*, **9**, 299–306.
- Lemos-Espinal, J.A. & Smith, H.M. (2007a) *Anfibios y reptiles del Estado de Chihuahua, México/Amphibians and reptiles of the State of Chihuahua, México*. CONABIO, Mexico, D.F.
- Lemos-Espinal, J.A. & Smith, H.M. (2007b) *Anfibios y reptiles del Estado de Coahuila, México/Amphibians and reptiles of the State of Coahuila, México*. CONABIO, Mexico, D.F.
- León-Paniagua, L., Navarro-Sigüenza, A.G., Hernández-Baños, B.E. & Morales, J.C. (2007) Diversification of the arboreal mice of the genus *Habromys* (Rodentia: Cricetidae):

- Neotominae) in the Mesoamerican highlands. *Molecular Phylogenetics and Evolution*, **42**, 653–664.
- Marshall, C.J. & Liebherr, J.K. (2000) Cladistic biogeography of the Mexican transition zone. *Journal of Biogeography*, **27**, 203–216.
- Martin, P.S. (1958) A biogeography of reptiles and amphibians in the Gómez Fariás region, Tamaulipas, México. *Miscellaneous Publications, Museum of Zoology, University of Michigan*, **101**, 1–102.
- McCormack, J.E., Peterson, A.T., Bonaccorso, E. & Smith, T.B. (2008) Speciation in the highlands of Mexico: genetic and phenotypic divergence in the Mexican jay (*Aphelocoma ultramarina*). *Molecular Ecology*, **17**, 2505–2521.
- McCormack, J.E., Joseph, H., Delaney, K.S., Peterson, A.T. & Knowles, L. (2010) Calibrating divergence times on species trees versus gene trees: implications for speciation history of the *Aphelocoma* jays. *Evolution*, **65**, 184–202.
- McCranie, J.R. & Wilson, L.D. (1987) The biogeography of the herpetofauna of the pine-oak woodlands of the Sierra Madre Occidental of Mexico. *Milwaukee Public Museum Contributions in Biology and Geology*, **72**, 1–30.
- McDonald, J.A. (1993) Phytogeography and history of the alpine–subalpine flora of northeastern Mexico. *Biological diversity in Mexico: origins and distribution* (ed. by T.P. Ramamoorthy, R. Bye, A. Lot and J. Fa.), pp. 681–703. Oxford University Press, New York.
- Metcalfe, S.E., O'Hara, S.L., Caballero, M. & Davies, S.J. (2000) Records of Late Pleistocene–Holocene climatic change in Mexico – a review. *Quaternary Science Reviews*, **19**, 699–721.
- Mittermeier, R.A., Gil, P.R., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreux, J. & da Fonseca, G.A.B. (2005) *Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions*. Conservation International, Washington, DC.
- Moore, W.S. (1995) Inferring phylogenies from mtDNA variation: mitochondrial gene trees versus nuclear gene trees. *Evolution*, **49**, 718–726.
- Morafka, D.J. (1977) A biogeographical analysis of the Chihuahuan Desert through its herpetofauna. *Biogeographica*, **9**, 1–313.
- Morrone, J.J. (2009) *Evolutionary biogeography: an integrative approach with case studies*. Columbia University Press, New York.
- Morrone, J.J. (2010) Fundamental biogeographic patterns across the Mexican Transition Zone: an evolutionary approach. *Ecography*, **33**, 355–361.
- Moruela-Holme, N., Fløjgaard, C. & Svenning, J.C. (2010) Climate change risks and conservation implications for a threatened small-range mammal species. *PLoS ONE*, **5**, e10360.
- Mulcahy, D.G. & Mendelson, J.R., III (2000) Phylogeography and speciation of the morphologically variable, widespread species *Bufo valliceps*, based on molecular evidence from mtDNA. *Molecular Phylogenetics and Evolution*, **17**, 173–189.
- Mulcahy, D.G., Morrill, B.H. & Mendelson, J.R., III (2006) Historical biogeography of lowland species of toads (*Bufo* across the Trans-Mexican Neovolcanic Belt and the Isthmus of Tehuantepec. *Journal of Biogeography*, **33**, 1889–1904.
- Murphy, R.W., Fu, J., Lathrop, A., Feltham, J.V. & Kovak, V. (2002) Phylogeny of the rattlesnakes (*Crotalus* and *Sistrurus*) inferred from sequences of five mitochondrial DNA genes. *Biology of the vipers* (ed. by G.W. Schuett, M. Höggren, M.E. Douglas and H.W. Greene), pp. 69–92. Eagle Mountain Publishing, Eagle Mountain, UT.
- Navarro-Sigüenza, A.G., Peterson, A.T., Nyari, A., García-Deras, G. & García-Moreno, J. (2008) Phylogeography of the *Buarremon* brush-finch complex (Aves, Emberizidae) in Mesoamerica. *Molecular Phylogenetics and Evolution*, **47**, 21–35.
- Nogués-Bravo, D. (2009) Predicting the past distribution of species climatic niches. *Global Ecology and Biogeography*, **18**, 521–531.
- Nylander, J.A.A. (2004) *MrModeltest v. 2. Program distributed by the author*. Evolutionary Biology Centre, Uppsala University, Uppsala. Available at: <http://www.abc.se/~nylander/>.
- Otto-Bliesner, B.L., Brady, E.C., Clauzet, G., Tomas, R., Levis, S. & Kothavala, Z. (2006) Last Glacial Maximum and Holocene climate in CCSM3. *Journal of Climate*, **19**, 2526–2544.
- Paniagua, L.L. & Morrone, J.J. (2009) Do the Oaxacan Highlands represent a natural biotic unit? A cladistic biogeographical test based on vertebrate taxa. *Journal of Biogeography*, **36**, 1939–1944.
- Pearson, R.G., Raxworthy, C., Nakamura, M. & Peterson, A.T. (2007) Predicting species' distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, **34**, 102–117.
- Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.
- Puebla-Olivares, F., Bonaccorso, E., Espinosa de los Monteros, A., Omland, K.E., Llorente-Bousquets, J.E., Peterson, A.T. & Navarro-Sigüenza, A.G. (2008) Speciation in the emerald toucanet (*Aulacorhynchus prasinus*) complex. *The Auk*, **125**, 39–50.
- Pyron, R.A. & Burbrink, F.T. (2009) Lineage diversification in a widespread species: roles for niche divergence and conservatism in the common kingsnake, *Lampropeltis getula*. *Molecular Ecology*, **18**, 3443–3457.
- Raes, N. & ter Steege, H. (2007) A null-model for significance testing of presence only species distribution models. *Ecography*, **30**, 727–736.
- Ramamoorthy, T., Bye, R., Lot, A. & Fa, J. (1993) *Biological diversity of Mexico: origins and distribution*. Oxford University Press, Oxford.
- Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.4*. Available at: <http://beast.bio.ed.ac.uk/Tracer>.
- Ree, R.H. & Smith, S.A. (2008) Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4–14.
- Riddle, B.R., Hafner, D.J., Alexander, L.F. & Jaeger, J.R. (2000) Cryptic vicariance in the historical assembly of a Baja California peninsular desert biota. *Proceedings of the National Academy of Sciences USA*, **97**, 14438–14443.

- Rissler, L.J. & Apodaca, J.J. (2007) Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology*, **56**, 924–942.
- Rissler, L.J., Hijmans, R.J., Graham, C.H., Moritz, C. & Wake, D.B. (2006) Phylogeographic lineages and species comparisons in conservation analysis: a case study of California herpetofauna. *The American Naturalist*, **167**, 655–666.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.
- Rosas-Elguera, J., Alva-Valdivia, L., Goguitchaichvili, A., Urrutia-Fucugauchi, J., Ortega-Rivera, M., Prieto, J. & Lee, J. (2003) Counterclockwise rotation of the Michoacan Block: implications for the tectonics of western Mexico. *International Geology Review*, **45**, 814–826.
- Salinas-Moreno, Y., Mendoza-Correa, G., Barrios, M.A., Cisneros, R., Macías-Sámamo, J. & Zúñiga, G. (2004) Areography of the genus *Dendroctonus* (Coleoptera: Curculionidae: Scolytinae) in México. *Journal of Biogeography*, **31**, 1163–1177.
- Schmidly, D. (1977) Factors governing the distribution of mammals in the Chihuahuan Desert region. *Transactions of the symposium on the biological resources of the Chihuahuan Desert region, United States and Mexico* (ed. by R.H. Wauer and D.H. Riskind), pp. 163–192. Sul Ross State University, Alpine, TX.
- Sullivan, J., Markert, J.A. & Kilpatrick, C.W. (1997) Phylogeography and molecular systematics of the *Peromyscus aztecus* group (Rodentia: Muridae) inferred using parsimony and likelihood. *Systematic Biology*, **46**, 426–440.
- Upton, D.E. & Murphy, R.W. (1997) Phylogeny of the side-blotched lizards (Phrynosomatidae: *Uta*) based on mtDNA sequences: support for a midpeninsular seaway in Baja California. *Molecular Phylogenetics and Evolution*, **8**, 104–113.
- Van Devender, T.R. & Burgess, T.L. (1985) Late Pleistocene woodlands in the Bolson de Mapimi: a refugium for Chihuahuan Desert biota? *Quaternary Research*, **24**, 346–353.
- Wiens, J.J., Parra-Olea, G., García-París, M. & Wake, D.B. (2007) Phylogenetic history underlies elevational patterns of biodiversity in tropical salamanders. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 919–928.
- Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2004) *AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference*. Available at: <http://ceb.csit.fsu.edu/awty> (accessed 24 November 2010).
- Wüster, W., Ferguson, J.E., Quijana-Mascareñas, J.A., Pook, C.E., Salomão, M.G. & Thorpe, R.S. (2005) Tracing an invasion: landbridges, refugia and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). *Molecular Ecology*, **14**, 1095–1108.
- Wüster, W., Peppin, L., Pook, C.E. & Walker, D.E. (2008) A nesting of vipers: phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). *Molecular Phylogenetics and Evolution*, **49**, 445–459.
- Zaldivar-Riverón, A., Nieto-Montes de Oca, A. & Lacleste, J.P. (2005) Phylogeny and evolution of dorsal pattern in the Mexican endemic lizard genus *Barisia* (Anguillidae: Gerrhonotinae). *Journal of Zoological Systematics and Evolutionary Research*, **43**, 243–257.
- Zink, R.M. & Barrowclough, G.F. (2008) Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, **17**, 2107–2121.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Putative highland species or species pairs cited by Martin (1958) with disjunct distributions in the highlands of the Sierra Madre Occidental and Sierra Madre Oriental, and either absent or replaced by sister taxa along the Transvolcanic Belt.

Appendix S2 (a) Collection and voucher data for genetic samples of highland rattlesnakes of the *Crotalus intermedius* group used in this study and deposited in the Royal Ontario Museum (ROM), and (b) GenBank accession numbers for outgroup samples used.

Appendix S3 Map showing geo-referenced localities of *Crotalus pricei* samples used in species distribution models in this study.

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Robert W. Bryson Jr and **Robert W. Murphy** began this collaborative research while R.W.B. was a Master's student at Sul Ross State University, building on their shared interests in rattlesnake systematics and the biogeography of Mexico. All authors are broadly interested in better understanding the biodiversity of Mexico through evolutionary (R.W.B., R.W.M., M.R.G., A.L.) and ecological (D.L.V.) studies.

Author contributions: R.W.B. and R.W.M. conceived the ideas; R.W.B., D.L.V., M.R.G. and A.L. collected the data; R.W.B., R.W.M., M.R.G. and A.L. analysed the data; and R.W.B. and R.W.M. led the writing.

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