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ORIGINAL ARTICLE

Phylogeography of the Arizona hairy scorpion (*Hadrurus arizonensis*) supports a model of biotic assembly in the Mojave Desert and adds a new Pleistocene refugium

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ABSTRACT

Aim As data accumulate, a multi-taxon biogeographical synthesis of the Mojave Desert is beginning to emerge. The initial synthesis, which we call the 'Mojave Assembly Model', was predominantly based on comparisons of phylogeographical patterns from vertebrate taxa. We tested the predictions of this model by examining the phylogeographical history of *Hadrurus arizonensis*, a large scorpion from the Mojave and Sonoran deserts.

Location Mojave and Sonoran deserts, United States and Mexico.

Methods We sequenced mitochondrial cytochrome *c* oxidase subunit I (*COI*) data from 256 samples collected throughout the range of *H. arizonensis*. We analysed sequence data using a network analysis, spatial analysis of molecular variance (SAMOVA), and a Mantel test. We then used a molecular clock to place the genetic patterns in a temporal framework. We tested for signals of expansion using neutrality tests, mismatch distributions and Bayesian skyline plots. We used Maxent to develop current and late-glacial species distribution models from occurrence records and bioclimatic variables.

Results Phylogenetic and structure analyses split the maternal genealogy basally into a southern clade along the coast of Sonora and a northern clade that includes six lineages distributed in the Mojave Desert and northern Sonoran Desert. Molecular dating suggested that the main clades diverged between the late Pliocene and early Pleistocene, whereas subsequent divergences between lineages occurred in the middle and late Pleistocene. Species distribution models predicted that the distribution of suitable climate was reduced and fragmented during the Last Glacial Maximum.

Main conclusions Genetic analyses and species distribution modelling suggest that the genetic diversity within *H. arizonensis* was predominantly structured by Pleistocene climate cycles. These results are generally consistent with the predictions of Pleistocene refugia for arid-adapted taxa described in the Mojave Assembly Model, but suggest that a northern area of the Lower Colorado River Valley may have acted as an additional refugium during Pleistocene glacial cycles.

Keywords
Biogeography, *COI*, Maxent, mitochondrial DNA, Quaternary, Scorpiones, Sonoran Desert, species distribution modelling.

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INTRODUCTION

The deserts of south-western North America were shaped by a complex history of landscape evolution through the Neogene due to tectonic activity associated with the junction of the Pacific and North American plate boundaries (e.g. Flesch *et al.*, 2000). Species inhabiting these deserts during this time not only endured physical changes in the Earth's surface, such as the formation of basins and mountain ranges due to extensions of the lithosphere, but also coped with repeated changes in climate, especially during the Pleistocene (Riddle & Hafner, 2006). As a result, the biodiversity and endemism of the North American deserts is greater than that of other natural ecosystems in North America (Mittermeier *et al.*, 2003), probably having been elevated by vicariance and adaptation in a topographically dynamic landscape.

Biogeographical studies within the arid regions of North America indicate that many desert organisms exhibit similar histories (Hafner & Riddle, 2011). While many early biogeographical studies focused on broader-scale patterns within and between the North American deserts (e.g. Riddle & Honeycutt, 1990; Riddle, 1995), data accumulating from multiple taxa provide prospects for addressing more intricate biogeographical histories within individual regions. Recently, Bell *et al.* (2010) conducted one such synthesis by comparing phylogeographical data from two species of *Xerospermophilus* (round-tailed ground squirrels) to similar studies of co-occurring taxa in the Mojave and Sonoran deserts. Their model (hereafter referred to as the 'Mojave Assembly Model') outlines a preliminary hypothesis for the historical assembly of the Mojave Desert biota, including parts of the adjacent Sonoran Desert. The model can be summarized as a history of geologically and climatically induced vicariance events between the late Neogene and Pleistocene, followed by post-glacial expansion and secondary contact (see Fig. 1 for a visual overview).

In short, the Mojave Assembly Model begins with diversification associated with the development of the Colorado River and an aquatic incursion of the Colorado and Gila rivers, called the 'Bouse Formation', between the late Miocene and early Pliocene (reviewed in Mulcahy *et al.*, 2006). During the late Pliocene, orogenesis of the Sierra Nevada and Transverse Ranges (Wakabayashi & Sawyer, 2001; Jones *et al.*, 2004; Warrick & Mertes, 2009; but see Henry, 2009 for a review of alternative geological reconstructions), as well as uplift of the western Mojave Desert (Cox *et al.*, 2003), may have then left some arid-adapted forms isolated in rain-shadowed basins where they diverged in allopatry. Climatic

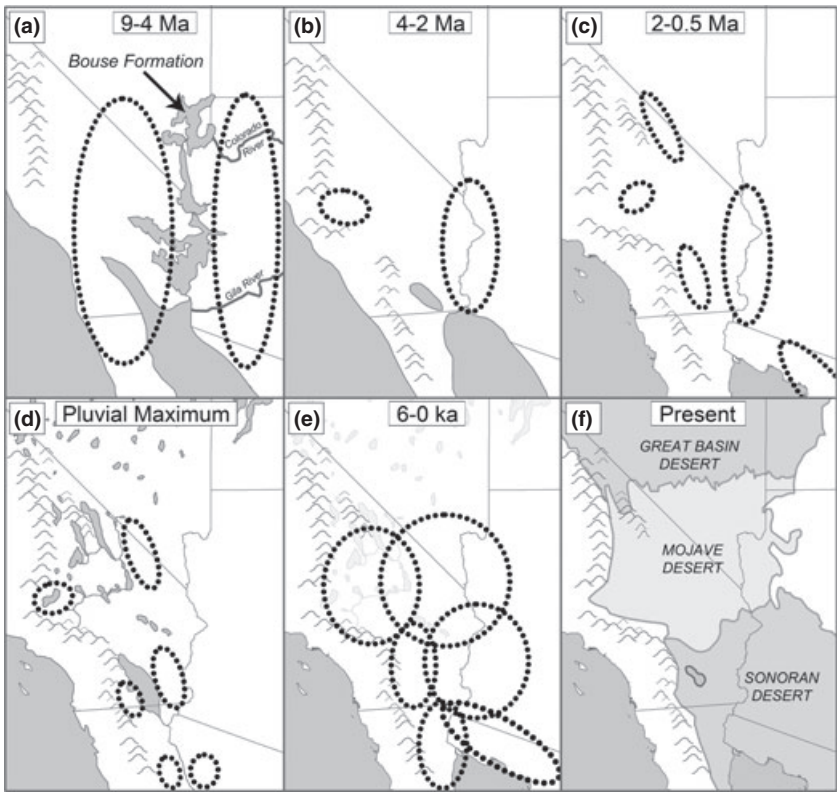

Figure 1 The 'Mojave Assembly Model' of historical assembly of the Mojave Desert biota: (a) distribution of taxa sundered by the Bouse Formation and development of a through-flowing Colorado River between 9 and 4 Ma; (b) distribution of taxa isolated in desert basins in the western Mojave Desert (Antelope and Phelan Peak basins) and along the Lower Colorado River Valley between 4 and 2 Ma; (c) location of taxa isolated in desert basins developing during the Pleistocene (2–0.5 Ma); (d) fragmented arid refugia during the late Pleistocene pluvial maximum; (e) expansion from arid refugia and secondary contact during the Holocene (6–0 ka); (f) current boundaries of the Mojave Desert and adjacent Sonoran and Great Basin deserts. Figure redrawn from Bell *et al.* (2010).

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conditions during Pleistocene glacial periods are thought to have further fragmented arid habitats, facilitating additional lineage formation associated with isolated desert basins, drainages, and other secluded regions of suitable climate. Following the Last Glacial Maximum (LGM), arid-adapted organisms then expanded their ranges out of the basins, with southern populations generally spreading northwards.

Support for the Mojave Assembly Model comes mostly from phylogeographical studies of terrestrial vertebrate taxa and, with the exception of *Homalonychus* spiders (Crews & Hedin, 2006), patterns proposed by the model have not been adequately assessed with terrestrial invertebrates. Herein, we contribute a detailed phylogeographical investigation of *Hadrurus arizonensis* Ewing, 1928, an arid-adapted scorpion distributed throughout low to mid-elevations of the Mojave and Sonoran deserts. This scorpion is most common in sandy areas such as dune systems and washes (Williams, 1970), where it constructs elaborate burrows up to 2 m in depth (Stahnke, 1966; Anderson, 1975). Also known as the Arizona hairy scorpion, *H. arizonensis* is the largest scorpion in North America (up to 127 mm in length). Colour patterns vary considerably across the range of this species (Williams, 1970), which may indicate phylogeographical structure. Three subspecies were formerly recognized based on these patterns (*Hadrurus arizonensis arizonensis* Ewing, 1928; *Hadrurus arizonensis austrinus* Williams, 1970; and *Hadrurus arizonensis pallidus* Williams, 1970), one of which (*H. a. pallidus*) was synonymized with the nominotypical subspecies when mitochondrial DNA (mtDNA) did not support morphological interpretations (Fet *et al.*, 2001).

We explored the phylogeography of *H. arizonensis*, with particular reference to the Mojave Assembly Model, using mtDNA sequence data from samples collected across its distribution in the Mojave and Sonoran deserts. We used species distribution modelling (Elith & Leathwick, 2009) to investigate changes in the distribution of climate suitable for *H. arizonensis* since the LGM (*c.* 21 ka). If *H. arizonensis* was influenced by events outlined by the Mojave Assembly Model, we would expect this invertebrate to yield phylogeographical patterns similar to those observed in co-distributed vertebrate species. Furthermore, if climatic conditions during Pleistocene glacial periods caused *H. arizonensis* to fragment into allopatric refugia, as predicted by the Mojave Assembly Model, species distribution models should depict a fragmented distribution during the LGM and genetic data should reveal evidence of lineage formation in areas where climates remained suitable.

MATERIALS AND METHODS

Taxon sampling

We collected samples in the field at night using ultraviolet lights (Stahnke, 1972). We obtained 256 samples from 84 unique localities (see Appendix S1 in Supporting Information). We removed legs from the left side of each individual scorpion and stored these tissues in 95% ethanol at −80 °C or preserved them in RNALater (Ambion, Austin, TX, USA) for DNA extraction. The remainder of each specimen was preserved as a voucher specimen and deposited at the American Museum of Natural History (AMNH) or the San Diego Natural History Museum (SDNHM), with those collected from Death Valley National Park on a long-term loan to SDNHM. We pooled localities less than 10 km apart and without obvious intervening biogeographical barriers for analyses, resulting in 64 general sites (Fig. 2, Appendix S1).

Molecular techniques

We sequenced a 1029-bp (base pair) fragment of the mitochondrial gene for cytochrome *c* oxidase subunit I (*COI*), which has previously been used in phylogeographical assessments of arachnids (e.g. Prendini *et al.*, 2003, 2005; Thomas & Hedin, 2008; Pfeiler *et al.*, 2009; Graham *et al.*, 2012). We isolated total genomic DNA from leg tissue using either a standard phenol–chloroform extraction or a DNeasy extraction kit (Qiagen, Valencia, CA, USA). We amplified the targeted gene by polymerase chain reaction (PCR) using ExTaq Polymerase Premix (Takara Mirus Bio, Madison, WI, USA) and combinations of external primers listed in Appendix S1. All combinations of external primers successfully amplified sequences at annealing temperatures ranging between 54 and 60 °C. As two regions of single nucleotide repeats (8–10 bp) caused signal strength at the 3′ end to weaken, we used internal primers to verify nucleotide calls in regions with weak signal by sequencing within the region amplified by the external primers. We conducted cycle sequencing using a BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Qiagen), and completed electrophoresis and visualization on an ABI 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA). We assembled sequences using Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and compared the sequences to a complete mtDNA sequence of *Uroctonus mordax* (GenBank no. EU523756.1). All *H. arizonensis* sequences were deposited in GenBank (accession numbers KC347040–KC347295).

Phylogenetic analyses and population structure

We assessed the *COI* phylogeny of *H. arizonensis* by Bayesian inference (BI), as implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), through the Cyberinfrastructure for Phylogenetic Research cluster (CIPRES Gateway 3.1) at the San Diego Supercomputer Center. We used the program Collapse 1.2 (http://tomato.biol.trinity.edu/programs/index.php/Collapse) to remove redundant haplotypes, and based our assessments on the unique haplotypes identified (see below). We then determined the best-fit models of nucleotide substitution for the haplotype data under several codon partitioning schemes (each position separately, positions 1+2

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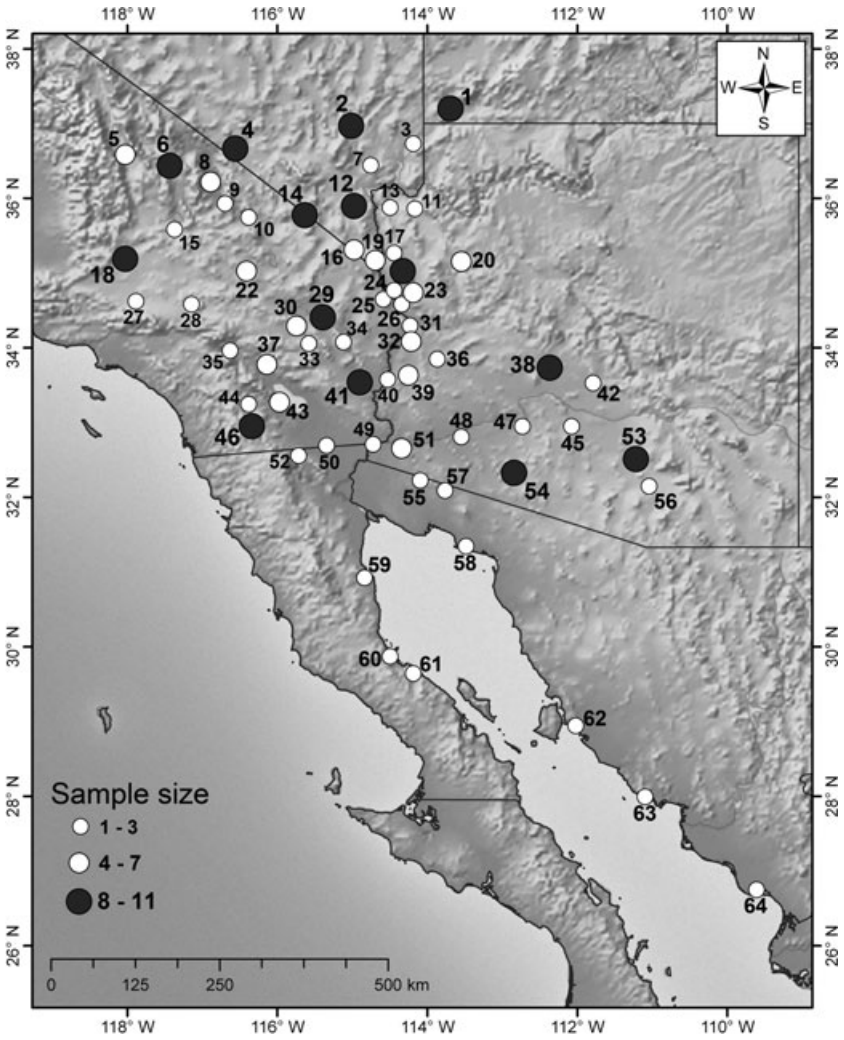

Figure 2 Map depicting locations for samples of *Hadrurus arizonensis* in south-western North America used in genetic analyses. Numbers correspond to locality data presented in Appendix S2. Filled circles indicate samples used in spatial analysis of molecular analysis (SAMOVA).

combined but position 3 separate, and unpartitioned) using jModelTest 0.1.1 and the Akaike information criterion (AIC; Posada, 2008). For the BI runs, model parameters were unlinked across character partitions, and we used the default parameters for the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) (three hot chains and one cold chain), except that we changed the heating parameter to 0.01 in order to keep state swap frequencies between 10% and 70%. We ran each partitioning scheme for 10 million generations, sampling trees every 1000 generations and discarding the first 25% as burn-in. All analyses were run twice, and after confirming that the duplicate Markov chains converged on similar mean likelihoods in Tracer 1.5 (Rambaut & Drummond, 2007) and the program awty (Nylander *et al.*, 2008), we inferred the best-fit partitioning scheme using Bayes factors (Nylander *et al.*, 2004). We based our final interpretations on the 50% majority-rule consensus tree and its associated posterior probabilities from the two runs of the best model. Although using only mtDNA for phylogenetic analyses is controversial, particularly when interpreting these data as estimates of species patterns (Edwards & Bensch, 2009), the use of mtDNA is satisfactory to provide an initial investigation of biogeographical patterns (e.g. Zink & Barrowclough, 2008; Barrowclough & Zink, 2009).

Much of the structure in the resulting BI phylogeny was shallow (see Results), so we used the program Network 4.5.1.6 (Fluxus Technology, Clare, Suffolk, UK) to construct median-joining networks of the mtDNA haplotypes (Bandelt *et al.*, 1999). We first constructed preliminary networks and explored the effect of different transition/transversion weighting schemes (all assessments produced nearly identical topologies). We constructed a final network with transitions/transversions weighted 3:1 and used the parsimony option to remove excessive links (Polzin & Daneshmand, 2003).

To conduct a spatial analysis of molecular variance (SAMOVA), we identified genetically distinct geographical groups without a priori groupings using samova 1.0 (Dupanloup *et al.*, 2002). We used sequence data from sites with sample sizes $\geq$ 4 (27 sites), but in a separate assessment used only sites with sample sizes $\geq$ 8 (14 sites) to ensure that changes in sample size did not produce substantially different results. Using 500 iterations per run, we conducted assessments with the number of partitions (*K*-value) rising from 2 to 13 (the maximum number of groupings given the 14 sites) for the

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$\geq$ 8 data set and from 2 to 20 (the maximum number of groupings allowed by the program) for the $\geq$ 4 data set. We evaluated trends in F_{CT}, a measure of the degree of differentiation between groups, to determine which *K*-value best represented groupings that were maximally differentiated and geographically homogeneous. As the interpretation of SAMOVA may be affected by isolation by distance (Dupanloup *et al.*, 2002), we used Alleles In Space 3.11 (Miller, 2005) to perform a Mantel test (Mantel, 1967) to evaluate correlation between geographical Euclidean distances and uncorrected pairwise (*p*) distances (using 1000 randomizations).

Demographic history

We used Arlequin 3.11 (Excoffier *et al.*, 2005) to estimate several genetic indices for groups that were indicated by the BI tree, haplotype network and SAMOVA. We estimated nucleotide diversity (π) and haplotype diversity (h), because in a comparative context these diversity indices can reveal patterns of past demographic expansion or constriction (Grant & Bowen, 1998; see Results). We also calculated Fu's F_S (Fu, 1997) and performed mismatch analyses (Rogers, 1995), to test for demographic or spatial expansion within predefined groups. We constructed Bayesian skyline plots (BSPs) using beast 1.5.4 (Drummond & Rambaut, 2007) to estimate the shape of population growth through time for each group. This assessment required estimation of best-fit substitution models for each group (as above), followed by beast runs of 20 million generations for each group except one (group I; see Results), which required two independent runs of 60 million generations in order to reach an ESS > 200. Demographic plots were visualized using Tracer.

Molecular dating

We ran beast on a reduced data set consisting of eight samples, selected to capture the deeper genetic structure within *H. arizonensis*, to estimate divergence dates between mtDNA groups. For this analysis, we estimated a best-fit substitution model for the unpartitioned sequences using jModelTest. We used an uncorrelated lognormal clock model because our analysis of intraspecific patterns was over a relatively short time-scale and substitution rates were not expected to be strongly autocorrelated among lineages (Drummond *et al.*, 2006). We used a mutation rate of 0.007 substitutions/site/million years, based on geological calibrations for the separation of island and mainland populations of a scorpion species (*Mesobuthus gibbosus*) from the Aegean region in the eastern Mediterranean (Gantenbein *et al.*, 2005). We selected a standard deviation of 0.003, thereby encompassing an alternative mutation rate based on 16S rDNA in scorpions (Gantenbein & Largiadèr, 2002) which is thought to evolve at a similar rate to *COI* (Gantenbein *et al.*, 2005). We ran beast for 40 million generations with a Yule tree prior and retained samples every 1000 generations. We again used Tracer to confirm stationarity of the Markov chain, as well as to determine the adequacy of the effective sample sizes (ESS > 200 for each estimated parameter).

Because the beast topology derived from the eight exemplar samples differed at the more recent nodes from that resulting from the BI analysis of the entire data set (see Results), we also used BSPs to estimate the time to the most recent common ancestor (TMRCA) for the groups identified by BI, the haplotype network and SAMOVA. We again used Tracer to ensure stationarity and to obtain TMRCA estimates.

Species distribution models

We assembled a data set of 267 occurrence points, representing 84 unique sampling localities and 183 additional locations from georeferenced museum specimens (AMNH, SDNHM, Smithsonian Institution and California Academy of Sciences) for species distribution modelling. We identified each specimen to species visually based on diagnostic morphological characters. Most of the museum records lacked coordinates, so we used Google Earth (http://earth.google.com/) to estimate latitude and longitude from information on voucher labels using standard georeferencing techniques. We excluded records with georeferencing errors $\geq$ 5 km so that the input records matched the spatial resolution of the modelling rasters (2.5 arc-minutes).

We constructed species distribution models using the program Maxent 3.3.2 (Phillips *et al.*, 2006), which is known to perform well in comparisons with other modelling approaches (Elith *et al.*, 2006). We screened 19 bioclimatic predictor layers representing current climatic trends, seasonality, and extremes of temperature and precipitation (Hijmans *et al.*, 2005) by assessing correlations among the different layers based on values from grid cells containing occurrence records (Peterson *et al.*, 2007). We selected from among the correlated layers (Pearson's correlation coefficient > 0.75), retaining layers representing quarterly climates rather than monthly climates, and precipitation during the coldest quarter rather than precipitation during the wettest quarter. The final predictor layers comprised the following 11 bioclimatic layers: Bio2, mean diurnal temperature range; Bio3, isothermality; Bio6, minimum temperature of the coldest month; Bio7, temperature annual range; Bio8, mean temperature of the wettest quarter; Bio10, mean temperature of warmest quarter; Bio11, mean temperature of the coldest quarter; Bio15, precipitation seasonality; Bio17, precipitation of the driest quarter; Bio18, precipitation of the warmest quarter; and Bio19, precipitation of the coldest quarter. We masked (clipped) the bioclimatic layers to ecoregions (Olson *et al.*, 2001) that contain occurrence records (Mojave Basin and Range, Sonoran Desert, Arizona/New Mexico Mountains, Sinaloa Coastal Plain, Baja Californian Desert) to improve model accuracy and reduce problems with extrapolation (Pearson *et al.*, 2002; Thuiller *et al.*, 2004; Randin *et al.*, 2006).

We ran Maxent using logistic output with default settings and random seeding. We tested the robustness of the models

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by cross-validation, dividing presence points into five groups and performing 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 model training (Nogués-Bravo, 2009). We applied the default method available in Maxent for determining the area under the receiver operating characteristic curve (AUC) to assess model performance.

We projected the models onto simulated climates for the LGM derived from the Community Climate System Model (CCSM; Otto-Bliesner *et al.*, 2006) and the Model for Interdisciplinary Research on Climate (MIROC; Hasumi & Emori, 2004) to explore the distribution of suitable habitat for *H. arizonensis* during glacial periods. Climatic suitability was displayed in ArcGIS by converting continuous Maxent outputs into binary grids using the maximum training sensitivity plus specificity threshold. This threshold balances errors of omission (sensitivity) with the fraction of the study area predicted as suitable habitat, which is used as a proxy for commission error (specificity), and has performed well in comparisons of various threshold criteria (Liu *et al.*, 2005; Jiménez-Valverde & Lobo, 2007).

RESULTS

Phylogenetic analyses and population structure

The 256 *COI* sequences obtained for *H. arizonensis* yielded 141 unique haplotypes containing 149 variable sites, 103 of which were parsimony-informative. Uncorrected *p*-distances ranged from 0.0% to 4.1%, with an average of 1.1%. Examination of chromatograms revealed no evidence of double peaks, indels, frameshifts or premature stop codons that would indicate co-amplification of nuclear mitochondrial pseudogenes (Bertheau *et al.*, 2011).

Bayes factors indicated that partitioning by each codon position provided the best fit, and substitution models selected under the AIC were as follows: first = HKY+G, second = HKY+G, third = HKY+I+G. The resulting majority-rule consensus tree, rooted at the mid-point, exhibited two strongly supported deeper nodes (Fig. 3) that formed geographically cohesive clades – a northern clade representing the majority of the samples distributed throughout the northern half of the range, and a southern clade along the coast of Sonora. The uncorrected *p*-distances between samples within the southern clade ranged from 0.8% to 2.4%, with an average of 1.6%; and up to 2.5% in the northern clade, with an average of 1.1%. Average uncorrected *p*-distance between the northern and southern clades was 3.4%. Both clades contained considerable phylogeographical structure, with numerous subclades (groups) strongly supported within the northern clade (identified as groups I–VI; Fig. 3). There was no statistical support for relationships between most groups, with the exception of groups II and III (Fig. 3).

The median-joining haplotype network (Fig. 4) revealed subnetworks, or groups, that mostly corresponded to the clades and subclades identified in the BI analyses. As in the BI analysis, the southern samples formed a distinct group of haplotypes, separated from the large group of northern haplotypes by 19 mutational steps. The southernmost sample was further removed from the southern group by 17 steps. The remaining samples comprised those identified as the northern clade in the BI tree and were highly structured. The largest group within the northern clade (group I) occupied a central position within the haplotype network and was distributed across the centre of the range, extending from the northern coast of the Gulf of California, north along the Lower Colorado River Valley, to the northernmost sites in Nevada and Utah. Several long branches within this group were further labelled as groups A–D (Fig. 4).

The SAMOVA results using different sample sizes yielded similar F_{CT} values and groupings, so for ease of presentation we limited results to the ≥ 4 data set because it includes more sites and represents a more thorough geographical sample. SAMOVA indicated a high degree of geographical structuring in the northern clade, as F_{CT} values continued to increase over the range of possible groups (Table 1). An asymptote was reached at about five groups ($K = 5$), which corroborated four of six groups identified within the northern clade in the BI and network analyses (Fig. 5a). At $K = 6$, SAMOVA identified a group from north of the Gila River near Phoenix, Arizona (Surprise, Arizona) that was strongly supported by both BI and network analyses (Fig. 5b). At $K = 7$, a group in the western Anza-Borrego Desert region (Salton Trough) was identified, which was also supported by the haplotype network (Fig. 4: group A), but the distinctiveness of this group was not supported by the BI analysis (Fig. 3).

The Mantel test revealed a correlation between geographical and genetic distances ($r = 0.49$, $P > 0.01$), indicating the potential for isolation by distance (IBD). In the presence of IBD, SAMOVA results may be skewed, as the analysis is expected to identify partitions that fall between the most widely spaced populations or the middle of the sampling areas (Dupanloup *et al.*, 2002). Instead of conforming to patterns expected under IBD, the partitions (as K increased until F_{CT} values reached an asymptote) identified geographically cohesive lineages supported by the BI and network analyses.

Demographic history

Each of the groups within the northern clade possessed values of π ranging from 0.836 to 1.0 and values of h ranging from 0.002 to 0.007 (Table 1). Fu's F_S was negative in all cases (Table 1), indicating deviations from mutation–drift equilibrium, as would be expected for populations that have undergone recent expansion or selection (Fu, 1997). Mismatch distributions were unimodal for groups I–IV (Appendix S2), indicating recent demographic expansion or selection (Rogers & Harpending, 1992). The distribution curves were multimodal for groups V and VI (Appendix S2),

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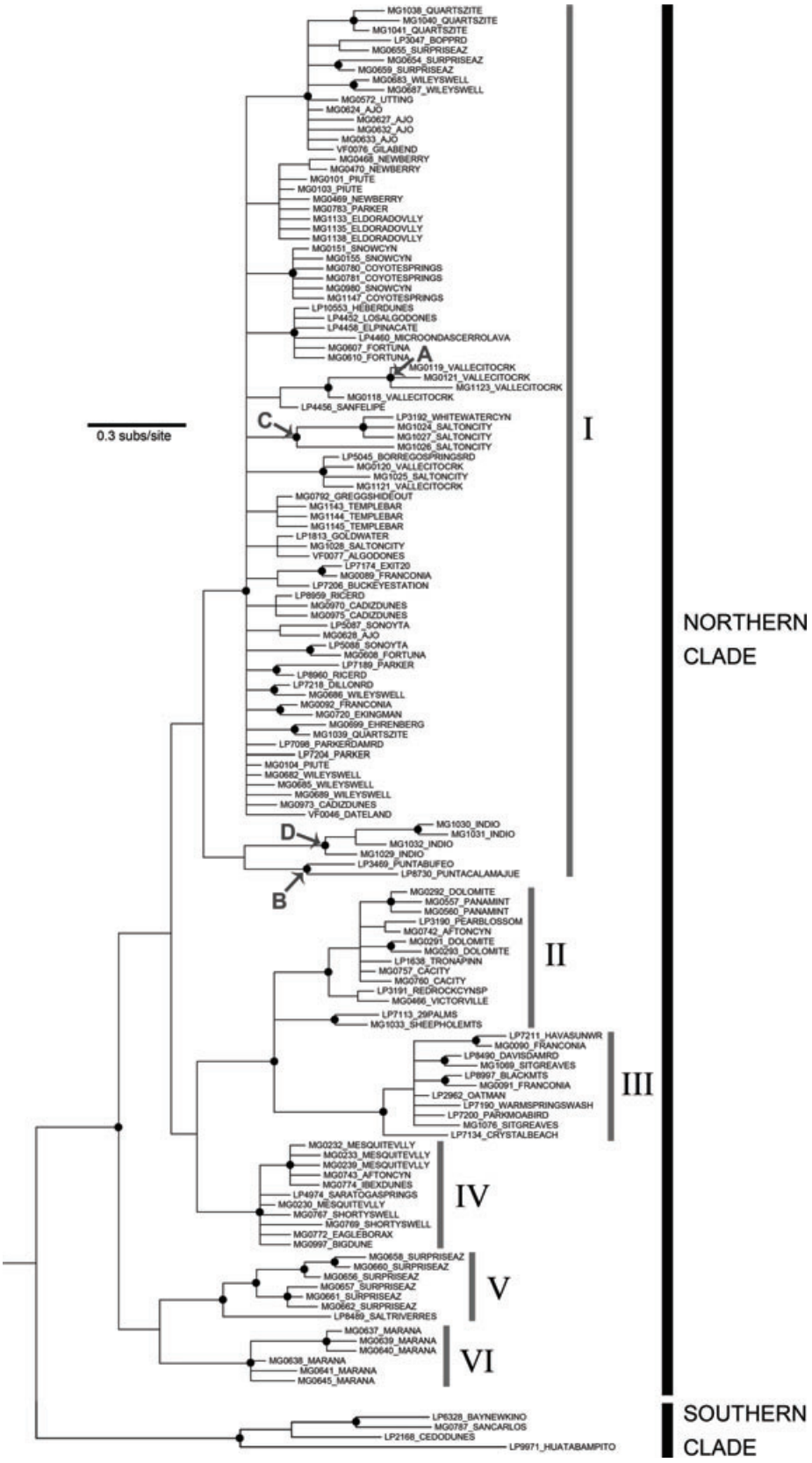

Figure 3 Midpoint-rooted consensus tree for *Hadrurus arizonensis* constructed using 1029 bp of *COI* mtDNA and estimated with Bayesian inference. Black circles indicate nodes supported with posterior probabilities of 0.9 or greater. Roman numerals represent groupings indicated by spatial analysis of molecular variance (SAMOVA). Letters A–D indicate subgroups identified in the haplotype network (Fig. 4).

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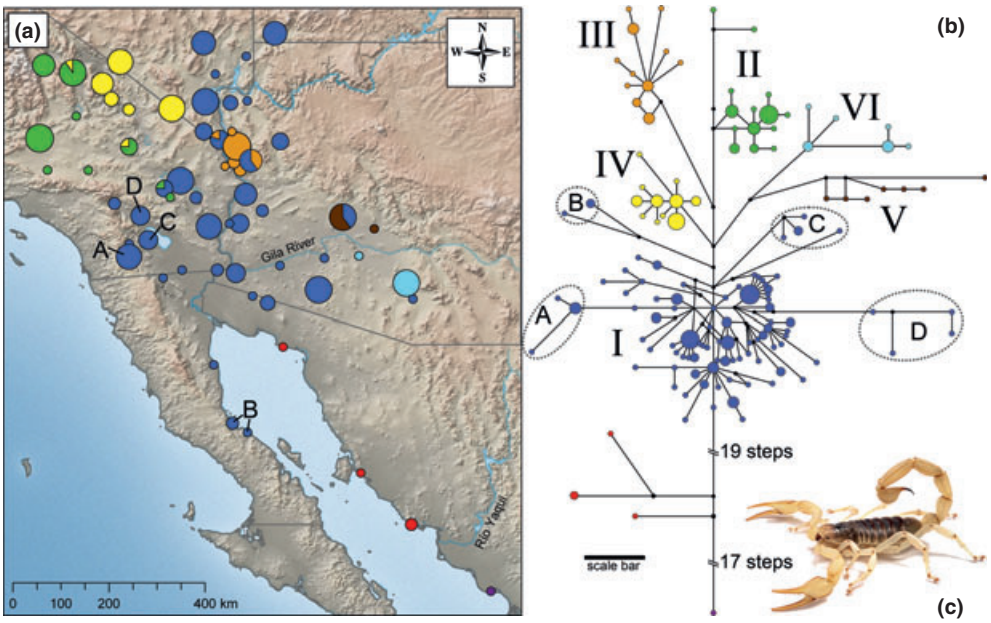

Figure 4 Map (a) and network (b) of mtDNA (*COI*) sequence haplotypes of *Hadrurus arizonensis* (c). Each circle in the network represents one haplotype. Circle size in both the map and network are proportional to sample size. Colours in the map correspond to the colours of each of the groups identified in the haplotype network. The scale bar is proportional to three transitions or one transversion. Roman numerals represent groupings indicated by spatial analysis of molecular variance (SAMOVA).

Table 1 Nucleotide diversity (π), haplotype diversity (h), Fu's F_S, results of mismatch analyses, and estimated time to most recent common ancestor (TMRCA, in Ma) for groups in the northern clade of *Hadrurus arizonensis* in south-western North America (see text). Asterisks indicate values with associated P-values < 0.02 for Fu's F_S (threshold value corresponding to $\alpha = 0.05$). Graphs of mismatch distributions are displayed in Appendix S2.

Group no.	n	π	h	F_S	Sudden expansion				Spatial expansion				Distribution curve	TMRCA (95% HPD)
					SSD	P	r	P	SSD	P	r	P		
I	146	0.007	0.982	−24.799*	0.001	0.444	0.007	0.007	0.001	0.642	0.007	0.716	Unimodal	1.02 (0.64–1.43)
II	32	0.002	0.841	−3.764*	0.008	0.212	0.057	0.387	0.008	0.172	0.057	0.424	Unimodal	0.58 (0.25–0.93)
III	21	0.003	0.910	−3.463*	0.004	0.772	0.017	0.952	0.006	0.701	0.017	0.951	Unimodal	0.31 (0.13–0.54)
IV	33	0.002	0.867	−4.630*	0.008	0.201	0.072	0.215	0.008	0.142	0.072	0.225	Unimodal	0.20 (0.006–0.39)
V	7	0.005	1.000	−2.987*	0.063	0.224	0.091	0.407	0.036	0.473	0.091	0.644	Multimodal	0.59 (0.27–0.96)
VI	11	0.003	0.836	−0.321	0.032	0.429	0.101	0.470	0.034	0.449	0.101	0.640	Multimodal	0.35 (0.12–0.63)

HPD, highest posterior density; SSD, sum of squared deviations.

suggesting that the populations may be at equilibrium, although sample sizes for both groups were low. Similarly, parametric bootstraps resulted in sum of squared deviations (SSD) that were all low, but much lower for groups I–IV. Raggedness values (r) were not significant for either the sudden expansion or spatial expansion mismatch models (Table 1), indicating that the data are a good fit for either model of expansion.

For group I, a history of moderate population growth during the late Pleistocene was depicted by the BSP (Appendix S2). This growth apparently ceased about 100,000 years ago when the population underwent a brief decline, followed by a period of rapid population growth and subsequent stability during the last 50 kyr. For all other groups, BSPs portrayed relatively stable population sizes through the late Pleistocene and Holocene.

Divergence dating

Divergence between the northern and southern clades was estimated from molecular dating (Appendix S2) to have occurred between the late Pliocene and mid-Pleistocene (3.08–1.79 Ma), with a mean estimate at the start of the Pleistocene (2.44 Ma). Divergence within the southern clade appears to have occurred between the early (2.4 Ma) and middle (1.03 Ma) Pleistocene. The TMRCA for each group in the northern clade (Table 1) was estimated to be between 1.43 Ma (Pleistocene) and 6 ka (Holocene).

Species distribution models

The species distribution models yielded high AUC scores for both training and testing data (both > 0.95), indicating that

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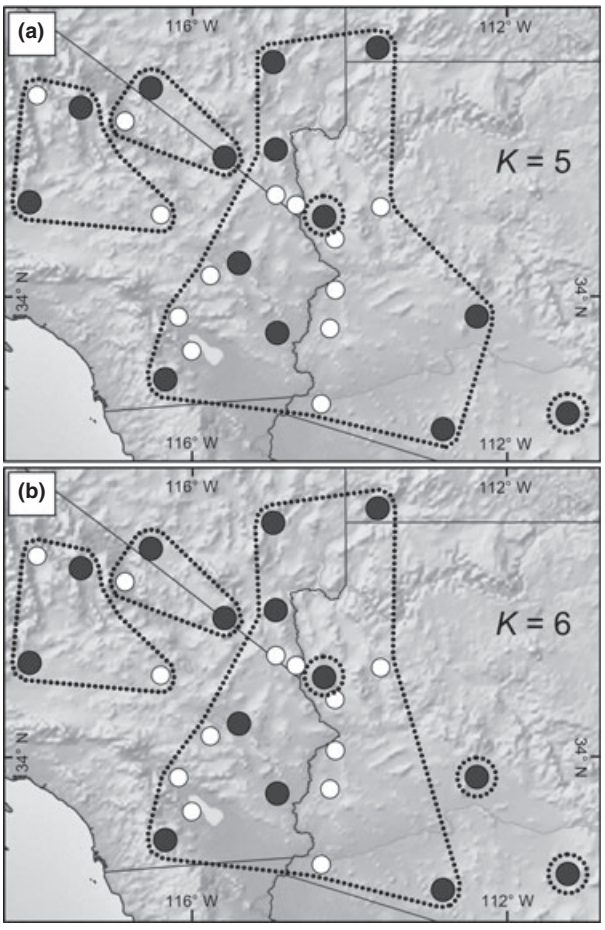

Figure 5 Results of spatial analysis of molecular variance (SAMOVA) for *Hadrurus arizonensis* in the Mojave and Sonoran deserts, with the number of partitions (K) set to five (a) and six (b). Large black circles represent samples used in the ≥ 8 data set, whereas both black and white circles indicate samples used in the ≥ 4 data set. Dotted lines indicate groups of populations that are geographically homogeneous and maximally differentiated. Roman numerals indicate groups also recovered in the network analyses (Fig. 4).

the models performed significantly better than random (Raes & ter Steege, 2007). The species distribution model under current climatic conditions (Fig. 6a) depicted largely contiguous suitable climate across the majority of the Mojave Desert and northern parts of the Sonoran Desert. Unsuitable areas were predicted in the mountainous regions of the Mojave Desert. In the south, climate was predicted to be suitable in a narrow region along the Mexican coastlines of Sonora and Baja California. The model appears to somewhat underestimate the distribution of suitable areas in Sonora, as two occurrence records in this region fall outside the predicted area.

LGM models based on different climatic scenarios (MIROC and CCSM) were similar (Fig. 6b,c), but incongruent along a northern portion of the Lower Colorado River Valley extending upriver to the mouth of the western Grand Canyon. Examination of multivariate environmental similarity surfaces (MESS) show slightly negative values for both models in this region, so the discrepancy could be due to 'novel' environments where at least one variable has a value outside the reference (current) range (Elith *et al.*, 2010). An area of even lower (more negative) MESS values occurred along the northern Sonora coast in the CCSM model, mostly driven by a variable representing average diurnal temperature range (Bio2). The CCSM model also predicted suitable but highly disjunct areas along the southern coast of Sonora and the northern coast of Baja California Sur, whereas the MIROC model did not.

Both models highlight at least two general areas that may have contained suitable climate during the LGM, one in the western Mojave Desert and another along the southern portion of the Colorado River. Although the degree of connectivity varied between models, both predicted suitable climate within low-elevation valleys of the western and north-western Mojave Desert. In the CCSM model, fragmented areas with suitable climates were predicted within Saline, Death and Panamint valleys, although these valleys were mostly filled in by Pleistocene lakes (Grayson, 1993). In contrast, the MIROC model predicted larger areas of suitable climate in these regions, suggesting that areas surrounding the Pleistocene lakes may have been suitable. The MIROC model predicted an area of suitable climate along the northern portion of the Lower Colorado River Valley, whereas the CCSM model did not. Southern areas predicted by both LGM models were similar, but extended further south in the MIROC model. The LGM models both highlighted southern portions of the Central Valley of California, an area currently occupied by the related species *Hadrurus obscurus*.

DISCUSSION

Phylogeography

Mitochondrial sequence data suggest that the phylogeography of *H. arizonensis* was shaped by a history of fragmentation, reduced gene flow and demographic expansion since the late Pliocene. Our assessments of phylogenetic and population structure (Figs 3–5) all identify two main clades: a southern clade distributed along the coast of Sonora and a widespread northern clade occupying the remainder of the distribution in the Sonoran and Mojave deserts. Neither clade corresponds with patterns of morphological variation used to distinguish the current (*H. a. arizonensis* and *H. a. austrinus*) or formerly recognized (*H. a. pallidus*; Fet *et al.*, 2001) subspecies, nor do the genetic patterns within each clade (discussed below).

Divergence between the two main clades appears to have occurred between the mid-Pliocene and early Pleistocene, a timeframe too recent to be explained by Neogene vicariance events such as the extensions of the Sea of Cortez (the Bouse Embayment) and development of the Colorado River (Fig. 1; reviewed in Wood *et al.*, 2008). Instead, divergence between the northern and southern clades could have been associated with contemporaneous uplift of the Transverse and Peninsu-

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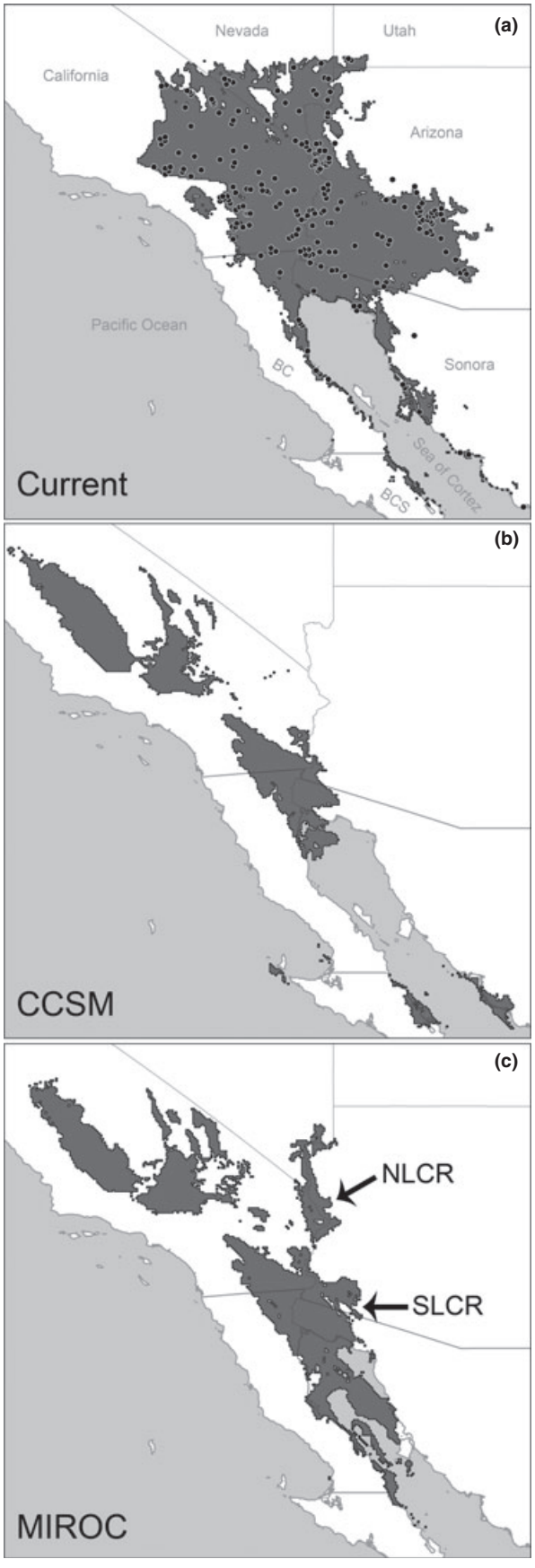

lar ranges, which created isolated rain-shadow deserts (Axelrod, 1979) where arid-adapted taxa are thought to have diverged in allopatry (Bell *et al.*, 2010). According to the Mojave Assembly Model, the first of these isolated basins developed during the Pliocene (between 4 and 2 Ma) – one located in the western Mojave Desert and another along the Lower Colorado River Valley (Fig. 1b). However, the split between the northern and southern clades probably occurred somewhere along the coast of Sonora, based on their distributions, so isolation within these basins was probably not responsible for the initial divergence between north and south. These clades are more likely to have diverged during the early Pleistocene, when desert taxa fragmented into additional desert basins (Bell *et al.*, 2010), including one along the coast of Sonora (Fig. 1c). A similar pattern was observed among clades of a cactophilic pseudoscorpion (*Dinocheirus arizonensis*) estimated to have diverged during a similar period, and which now appear to be in secondary contact near the boundary of the northern and southern clades of *H. arizonensis* (Pfeiler *et al.*, 2009).

Within the southern clade, levels of genetic differentiation between specimens were high, as the southernmost sample was 2.4% divergent (uncorrected *p*-distance) from the nearest coastal sample, 200 km to the north-west. Molecular dating placed this level of divergence between the early and mid-Pleistocene. Although our sampling in the south was sparse, genetic differentiation within the southern clade might be attributed to vicariance associated with the increased influence of the Río Yaqui (indicated in Fig. 4a). This river, like other rivers to the south, runs west from the Sierra Madre Occidental and has been postulated as a cause of genetic divergences in other taxa (Hafner & Riddle, 2011). The CCSM model suggests that LGM climates may have been suitable in a disjunct area on the southern Sonora coast (Fig. 6b). Persistence in this area during the LGM would explain the high genetic diversity in the southern clade, if individuals from this area have retained the genetic signal of an earlier divergence. Following the LGM, habitat may have become available along the rest of the Sonoran coast, allowing haplotypes north of the Río Yaqui to colonize new areas, following the coastline northwards. This scenario contradicts predictions from the MIROC model, which portrays no suitable climate along the southern and central coast of Sonora

Figure 6 Graphical results from species distribution models for *Hadrurus arizonensis* in south-western North America, generated using Maxent and displayed using the maximum training sensitivity plus specificity threshold. Models represent climate predicted as suitable (dark shading) during current conditions (a) and two Last Glacial Maximum (LGM) conditions estimated from CCSM (b) and MIROC (c) climatic simulations. Black dots (a) represent occurrence records used to generate the models. Arrows indicate postulated glacial refugia discussed in the text. Note that areas predicted as suitable in the Sea of Cortez during the LGM were terrestrial at the time. NLCR = northern Lower Colorado River Valley refugium, SLCR = southern Lower Colorado River Valley refugium.

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(Fig. 6c). Instead, suitable climate is depicted along the Lower Colorado River Valley and in fragmented patches that are currently within the Sea of Cortez, but were terrestrial during the LGM. Unfortunately, our genetic sampling was insufficient to decipher the biogeographical patterns in this region.

Phylogeographical patterns within the northern clade appear to have been shaped during the Pleistocene. Six geographically structured groups (Fig. 4b), representing monophyletic maternal lineages, were recovered by the phylogenetic and structure analyses (Figs 3–5), and molecular dating estimates placed the TMRCA for these groups in the mid-Pleistocene to early Holocene. Most of the mtDNA lineages within the northern clade of *H. arizonensis* are geographically congruent with glacial refugia predicted by the Mojave Assembly Model. However, two groups from the eastern portion of the species' range (groups V and VI) were not predicted by the Mojave Assembly Model (see 'Testing the Mojave Assembly Model' below). Demographic evidence also indicates that some populations of these scorpions underwent recent spatial expansions, as would be expected for arid-adapted taxa expanding their ranges as the climate warmed. The evidence of expansion is particularly strong for groups I–IV (Table 1, Appendix S2). Additional tests of demographic expansion conducted by comparing within-group values of π and h, based on the method employed by Grant & Bowen (1998), provide further evidence of expansion. Groups II–VI all have low π and high h, containing few highly divergent haplotypes. Such a genetic pattern would be expected under a hypothesis of Pleistocene fragmentation where populations underwent bottlenecks (such as contraction into glacial refugia), followed by rapid population growth and accumulation of novel mutations (Grant & Bowen, 1998). Genetic patterns within groups I and V show high π and high h, and appear to have had more stable population sizes through time.

Our results provide convincing evidence that the phylogeography of *H. arizonensis*, like co-occurring vertebrate species, was influenced by climatic fluctuations during the Pleistocene. As predicted by the Mojave Assembly Model, climatic conditions during glacial periods appear to have forced the northern distribution of *H. arizonensis* to fragment into several isolated regions mostly associated with desert basins and drainages. The isolation was sufficiently long, or recurrent enough, to establish reciprocally monophyletic mtDNA lineages. The only pattern that conflicts with the Mojave Assembly Model is that of an additional refugium along the northern section of the Lower Colorado River Valley, discussed below.

Northern Lower Colorado River Valley refugium

The phylogeography of *H. arizonensis* highlights an additional potential refugium that may have existed along a northern part of the Lower Colorado River Valley (hereafter referred to as the NLCR). Evidence for this refugium comes both from the presence of a geographically cohesive mtDNA lineage of *H. arizonensis* (Group III) in the area (Figs 3–5), and from one of the species distribution models which predicts that climate within the area was suitable for *H. arizonensis* during the LGM (Fig. 6c). The Group III haplotypes cluster within the centre of the area predicted by the model, but peripheral localities on the east and west of the predicted area also contain haplotypes from Group I. Furthermore, only Group I haplotypes were found in the northern part of the area predicted by the model, so the NLCR might have been contained within a smaller region than predicted. The CCSM model does not depict suitable climate in this region at all, so neither model may be entirely accurate.

Group I haplotypes found in sympatry with, and north of, the Group III haplotypes exhibited low haplotype diversity in relation to Group I haplotypes to the south, suggesting they may have recently expanded northwards from a larger refugium in the southern part of the Lower Colorado River Valley. This pattern raises an interesting question, namely why was the northern Mojave Desert not colonized by post-glacial expansion of haplotypes from the NLCR rather than by those from populations further south? Given that Group I and Group III haplotypes in this region occur on both sides of the Colorado River, this landscape feature clearly has not been a consistent barrier. In addition, there are no other obvious topographical barriers that might have influenced these distributions. One possibility is that there are differences in fitness between these lineages. Given the potential disparity between the sizes of the two refugial areas (Fig. 6), the population in the south may have experienced a wider range of ecological conditions than that within the NLCR during glacial periods. As the glacial periods lasted longer than the interglacials, the ecological requirements (fundamental niche) of fragmented populations may have diverged, yielding differential abilities to colonize the post-glacial landscape.

More intriguing is the possibility that dispersal or fitness differences between these lineages may result from potential hybridization of populations of *H. arizonensis* at sites within the NLCR with *Hadrurus spadix*, a closely related species that generally occurs at higher latitudes and elevations in the Mojave and Great Basin deserts. We found *H. arizonensis* and *H. spadix* in sympatry in the Newberry Mountains of Nevada and the Black Mountains of Arizona, both of which are areas within the NLCR. We observed specimens from the Newberry Mountains that appeared morphologically intermediate for diagnostic characters, lending support to the possibility of hybridization. A definitive assessment would, however, require an analysis of nuclear genes.

The presence of a Pleistocene NLCR is supported by phylogeographical patterns in some other taxa inhabiting the region. For example, the relict leopard frog (*Rana onca* = *Lithobates onca*) appears to have diverged from its closest relative, the lowland leopard frog [*Rana* (*Lithobates*) *yavapaiensis*], in this region during the Pleistocene, rendering the former narrowly distributed along river drainages within the NLCR and the latter distributed more broadly across the Sonoran Desert

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(Oláh-Hemmings *et al.*, 2010). Species distribution models constructed for the related scorpion species *H. spadix* from the Mojave and Great Basin deserts predict little suitable habitat in the Great Basin during the LGM, but suitable climate within the Mojave Desert includes a patch that encompasses the NLCR (M.R.G., unpublished data). Furthermore, species distribution models for the chisel-toothed kangaroo rat (*Dipodomys microps*), a rodent species endemic to the Great Basin Desert, predicted that LGM climates were suitable in an area very closely matching that of the NLCR (Jezkova *et al.*, 2011, their figure 4b). Therefore, the NLCR might represent an area where several Mojave and Great Basin desert species were able to persist during Pleistocene glacials.

Testing the Mojave Assembly Model

Except for the NLCR, phylogeographical patterns in *H. arizonensis* are mostly congruent with patterns predicted by late Pliocene to Holocene portions of the Mojave Assembly Model. During the late Pliocene to early Pleistocene, the Mojave Assembly Model predicts lineage formation in the western Mojave Desert as regional uplift forced desert organisms into basin refugia in the rain shadow of the Transverse Ranges. Although we recovered a unique lineage of *H. arizonensis* in this area (Group II), the molecular clock estimate placed the TMRCA for this group in the middle to late Pleistocene (0.25–0.93 Ma). During the Pleistocene, the Mojave Assembly Model posits that continued block-faulting formed the modern basin topography which, along with more mesic conditions, may have fragmented populations into basins associated with the Mojave River, Amargosa River, Salton Sea and Lower Colorado River (Fig. 1c). The distributions of groups I, II and IV from the northern clade suggest that *H. arizonensis* may also have occupied these areas during glacial periods. The centre of the distribution of Group I, the largest group, extends along the Lower Colorado River Valley and, as mentioned above, may have recently expanded from the southern part of this region. Group II is distributed throughout the western Mojave Desert, whereas the distribution of Group IV circumscribes the Amargosa River and Death Valley regions.

Demographic analyses suggest that groups II and IV underwent recent expansions (Table 1, Appendix S2), perhaps expanding their ranges from two small glacial refugia in the north-western Mojave Desert, as predicted by the Mojave Assembly Model. Bell *et al.* (2010) attributed these refugia to the Mojave and Amargosa River drainages. Species distribution models for *H. arizonensis*, however, do not predict suitable climate along the Amargosa River during the LGM, although adjacent low elevations of Death Valley are predicted as suitable (Fig. 6). During pluvial periods, Death Valley was filled with a large body of water known as Lake Manly, so although the climate may have been suitable, much of the predicted habitat almost certainly was not. Even so, the MIROC model predicted suitable LGM climate in an area larger than high-stand estimates for Lake Manly, so populations of *H. arizonensis* in Death Valley could have persisted in areas distributed around the lake, especially in sandy lakeshore habitats.

The Mojave Assembly Model predicts that Pleistocene climates may also have facilitated vicariance among desert organisms near the Gila River in Arizona. Although our sampling in this area was limited, unique groups of *H. arizonensis* haplotypes (groups V and VI) from the eastern part of its range also appear to be divided north and south of the Gila River. Given the relatively small size and ephemeral nature of this river, its significance as a biogeographical barrier is hard to imagine, but distinct groups of haplotypes have also been found on opposite sides of the river in *Phrynosoma platyrhinos* (desert horned lizards; Jones, 1995; Jezkova, 2010) and *Chaetodipus penicillatus* (desert pocket mice; Jezkova *et al.*, 2009). Jones (1995) attributed divergence in *P. platyrhinos* to a Pliocene inundation of the Colorado and Gila rivers during the Bouse Embayment (see Fig. 1a). However, Jezkova *et al.* (2009) estimated the genetic divergence in *C. penicillatus* to have occurred more recently, possibly by fragmentation of habitat in the Gila River area caused by Pleistocene climatic fluctuations, and not directly by the river itself. A similar situation could have occurred in *H. arizonensis*, as estimates from molecular dating also suggest that groups across the Gila River diverged during the Pleistocene (Table 1, Appendix S2).

The evidence of substantial genetic structure within the eastern part of the distribution of *H. arizonensis* suggests the potential for some degree of persistence within this region, especially during the LGM. This conflicts with the species distribution models that do not depict any suitable climate in the area during the LGM, instead suggesting that the nearest habitat was along the Lower Colorado River Valley. If the species distribution models are reasonably accurate in this region and *H. arizonensis* persisted in these eastern areas during the LGM, as implied by the genetic patterns, then populations there must have endured climate conditions outside of the current realized niche, and perhaps underwent 'niche drifting' (Jezkova *et al.*, 2011).

In summary, the phylogeography of *H. arizonensis* lends support to the predictions of the Mojave Assembly Model for distributional responses of arid-adapted organisms to Pleistocene climate change. Genetic diversity within *H. arizonensis* appears to have been influenced by climate-induced fragmentation and contraction into glacial refugia, which seems to have caused autochthonous lineage formation within specific regions throughout the Mojave and Sonoran deserts. Furthermore, an additional glacial refugium along the northern part of the Lower Colorado River (the NLCR) should be incorporated into future models for the historical assembly of the Mojave and Sonoran desert biotas.

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SUPPORTING INFORMATION

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

Appendix S1 Collection data (Table S1), primers (Table S2), and SAMOVA results (Table S3) from a phylogeographical assessment of *Hadrurus arizonensis*.

Appendix S2 Graphical results from demographic analyses (Fig. S1) and divergence dating (Fig. S2) from a phylogeographical assessment of *Hadrurus arizonensis*.

BIOSKETCH

Matthew R. Graham is interested in the biogeographical history of North America, especially from the perspective of terrestrial arthropods and herpetofauna. This study was part of his PhD research on scorpion phylogeography in the North American Southwest.

Author contributions: M.R.G., J.R.J. and B.R.R. collaborated on the study concept and design; M.R.G. collected and sequenced the majority of the samples and analysed the data; J.R.J. assisted with sample collection; J.R.J. and B.R.R. provided material support, while L.P. provided important samples, sequence data and primers. All authors contributed to the interpretation of analyses and to the writing, with M.R.G. leading and J.R.J. contributing substantially.

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