Molecular Phylogenetics and Evolution 69 (2013) 502–513

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeography of Beck's Desert Scorpion, *Paruroctonus becki*, reveals Pliocene diversification in the Eastern California Shear Zone and postglacial expansion in the Great Basin Desert

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ARTICLE INFO

Article history:
Received 12 November 2012
Revised 10 July 2013
Accepted 29 July 2013
Available online 9 August 2013

Keywords:
Biogeography
Basin and range
COI
Ecological niche modeling
Mitochondrial DNA
Mojave Desert

ABSTRACT

The distribution of Beck's Desert Scorpion, *Paruroctonus becki* (Gertsch and Allred, 1965), spans the 'warm' Mojave Desert and the western portion of the 'cold' Great Basin Desert. We used genetic analyses and species distribution modeling to test whether *P. becki* persisted in the Great Basin Desert during the Last Glacial Maximum (LGM), or colonized the area as glacial conditions retreated and the climate warmed. Phylogenetic and network analyses of mitochondrial cytochrome *c* oxidase 1 (*cox1*), 16S rDNA, and nuclear internal transcribed spacer (ITS-2) DNA sequences uncovered five geographically-structured groups in *P. becki* with varying degrees of statistical support. Molecular clock estimates and the geographical arrangement of three of the groups suggested that Pliocene geological events in the tectonically dynamic Eastern California Shear Zone may have driven diversification by vicariance. Diversification was estimated to have continued through the Pleistocene, during which a group endemic to the western Great Basin diverged from a related group in the eastern Mojave Desert and western Colorado Plateau. Demographic and network analyses suggested that *P. becki* underwent a recent expansion in the Great Basin. According to a landscape interpolation of genetic distances, this expansion appears to have occurred from the northwest, implying that *P. becki* may have persisted in part of the Great Basin during the LGM. This prediction is supported by species distribution models which suggest that climate was unsuitable throughout most of the Great Basin during the LGM, but that small patches of suitable climate may have remained in areas of the Lahontan Trough.

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1. Introduction

The Great Basin Desert is perhaps the most biologically unique of the currently recognized North American deserts (Hafner and Riddle, 2011). The region is positioned between the Sierra Nevada to the west and the Rocky Mountains to the east, and occurs further north and at a higher elevation than the other North American deserts (Cronquist et al., 1972; Olson et al., 2001). These factors together make the summer climate less severe, and the winters longer and colder. Accordingly, the Great Basin is sometimes referred to as the only 'cold' or 'temperate' desert in North America (Grayson, 1993).

During the late Pleistocene, the Great Basin climate was at times even colder, with temperatures fluctuating between cool glacial periods and warm interglacials (Spaulding, 1990; Thompson, 1990). Such climatic fluctuations probably made conditions even more extreme for desert organisms. Based on macrofossil data from packrat middens, many Great Basin plants are thought to have undergone severe range shifts, especially since the Last Glacial Maximum (LGM approximately 21 Kya), in response to Pleistocene climate fluctuations (Thompson, 1990; Thompson and Anderson, 2000). Although arid shrub-steppe vegetation was able to persist in some northern areas during the most recent Pleistocene glacial-interglacial cycles (Madsen et al., 2001; Wilson and Pitts, 2010), phylogeographic data suggest that several arid-adapted taxa may have colonized the Great Basin only recently, following the LGM (Nowak et al., 1994; Hornsby and Matocq, 2011). Other arid-adapted species, however, appear to have remained in the Great Basin during the Pleistocene in spite of severe habitat changes. These species potentially endured fluctuations in climate by shifting realized niches (within more stable fundamental niches), a phenomenon referred to as 'niche drifting' (Jezkova et al., 2011). Furthermore, genetic data and species distribution models (SDMs) suggest that some montane species in the Great Basin responded to Pleistocene climates by shifting ranges along elevational gradients (Walteri and Guralnick, 2008; Galbreath et al., 2009, 2010), colonizing lower elevations during glacial periods and higher elevations during interglacials. Information from

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1055-7903/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ympev.2013.07.028

fossils, phylogenetics, and SDMs all portray an extensive resorting of plant and animal communities within the Great Basin as the climate changed between the LGM and the current interglacial.

At the southern end of the Great Basin, phylogeographic datasets are beginning to reveal the biogeographical history of the Mojave Desert (Bell et al., 2010). Occurring at lower latitudes and lower mean elevations, the Mojave experiences more extreme summers than the Great Basin, but the winters are less severe (Redmond, 2009). The Mojave contains flora and fauna from many of the surrounding regions with few endemics, making it a transitional desert between the cooler Great Basin to the north and the warmer Peninsular and Sonoran deserts to the south (Bell et al., 2010; Hafner and Riddle, 2011). During glacial extremes, the geographical distributions of many arid-adapted organisms in the Mojave are thought to have contracted into areas associated with desert basins and drainages, where some were isolated long enough to form distinct lineages (see review in Bell et al., 2010). In the northern Mojave, there is also evidence that some regions harbored arid-adapted taxa that are now primarily distributed throughout the Great Basin (Jezkova et al., 2011).

The Beck's Desert Scorpion *Paruroctonus becki* (Gertsch and Allred, 1965) occurs in both the Mojave and Great Basin. We investigated the phylogeography of this common, arid-adapted scorpion to better understand how organisms with distributions spanning these deserts responded to climatic warming since the LGM. Our primary aim was to test whether *P. becki* persisted in the Great Basin during the LGM, or colonized the area relatively recently as glacial conditions retreated and climates warmed. Our secondary aim was to assess whether phylogeographic patterns among *P. becki* populations might reveal aspects about lineage diversification in the Mojave.

To accomplish these objectives, we sequenced a portion of the mitochondrial gene cytochrome *c* oxidase 1 (*cox1*), from samples collected throughout the range of *P. becki.* In an attempt to further resolve phylogenetic patterns derived from the *cox1* data, we also sequenced a portion of 16S ribosomal RNA (16S) from a subset of samples that characterized the majority of the *cox1* variation (exemplars from the major *cox1* groups), producing a larger dataset when concatenated with the *cox1* data. We then assessed the matrilineal genealogy within a temporal context using a relaxed molecular clock, and used demographic analyses to compare the genetic data to expectations under models of population expansion. Relying solely on mitochondrial DNA (mtDNA) for evolutionary reconstructions is controversial (e.g., Zink and Barrowclough, 2008; Edwards and Bensch, 2009). We therefore sequenced a variable nuclear gene, the internal transcribed spacer region (ITS-2), from a subset of individuals. In addition, we constructed climate-based species distribution models to examine the potential distribution of suitable climate for *P. becki* during the LGM for comparison with our interpretations derived from genetic data.

Phylogeographic studies often expose previously overlooked patterns in morphological variation among populations, sometimes with important taxonomic implications (e.g. Fouquet et al., 2007; Wood et al., 2008; Köhler and Glaubrecht, 2010). We discuss our results in light of current taxonomic hypotheses for *P. becki* and two morphologically similar species from California, *Paruroctonus variabilis* Hjelle and *Paruroctonus silvestrii* Borelli.

2. Materials and methods

2.1. Taxon sampling

Using ultraviolet light detection (Stahnke, 1972), we collected 89 samples of *P. becki* from 51 locations throughout the Mojave and Great Basin (Fig. 1; Table 1). Four specimens representing three congeners were included as outgroups: *Paruroctonus boreus* (Girard), *P. silvestrii*, and *P. variabilis*. Legs were removed from each specimen for DNA isolation and stored in 95% ethanol at −80°, with the vouchers retained in 70% ethanol. All tissues and vouchers were accessioned at the American Museum of Natural History.

2.2. Molecular techniques

We isolated genomic DNA from leg tissues by using either a standard phenol-chloroform extraction or a DNeasy Extraction Kit (Qiagen Inc., Valencia, CA, USA). We sequenced a fragment of the *cox1* gene using the primers LE1r (Esposito, 2011) and COImodF (Bryson et al., 2013). This gene was chosen because it has proven useful in intraspecific studies of scorpions (Prendini et al., 2003; Graham et al., 2013) and other arachnids (Prendini et al., 2005; Thomas and Hedin, 2008; Wang et al., 2008). Based on a preliminary assessment of the *cox1* dataset (see Results), a subset of samples representing most of the genetic structure observed in this gene was selected for additional sequencing. Fragments of 16S were sequenced using primers from Gantenbein et al. (1999) and nuclear ITS-2 using primers from Ji et al. (2003).AmpliTaq Gold (Applied Biosystems, Inc., Foster City, CA, USA) was used for polymerase chain reactions, with *cox1* fragments amplified at 50–54 °C for 34 cycles, 16S at 50 °C for 30 cycles, and ITS-2 at 50 °C for 35 cycles. Fluorescence-based cycle sequencing was conducted using the PCR primers (Appendix B) and a BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Qiagen Inc., Valencia, CA, USA), with electrophoresis on an ABI 3130 automated sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sequences were edited using SEQUENCHER v. 4.9 (Gene Codes Corp., Inc., Ann Arbor, MI, USA) and DnaSP v. 5 (Librado and Rozas, 2009). All sequences were deposited in GenBank (accession numbers KF548316–KF548479).

2.3. Phylogenetics and divergence dating

We assessed phylogenetic patterns using Bayesian inference (BI) implemented in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003) on the Cyberinfrastructure for Phylogenetic Research cluster (CIPRES Gateway v 3.1) at the San Diego Supercomputer Center. Best-fit models of nucleotide substitution were determined for the *cox1* haplotype data under several codon partitions (each codon position separately, positions 1 + 2 and 3 separate, and unpartitioned) using jModeltest v. 0.1.1 with the Akaike information criterion (Posada, 2008). Substitution models calculated for different gene partitions were also explored for a concatenated mtDNA dataset (*cox1* + 16S). A best-fit partitioning scheme for each dataset was then determined by assessing Bayes factors on the harmonic mean marginal likelihood values (Nylander et al., 2004). Our biogeographical interpretations were based on final runs of 10 million generations using the appropriate partitioning scheme and substitution models. Trees were sampled every 1000 generations with the first 2.5 million generations discarded as burn-in after confirming chain stationarity using TRACER v. 1.5 (Rambaut and Drummond, 2007) and AWTY (Nylander et al., 2008).

We used the parsimony option (Polzin and Daneshmand, 2003) in NETWORK v. 4.5.1.6 (Fluxus Technology Ltd. 2004) to construct separate median-joining networks of mtDNA and nuclear haplotypes (Bandelt et al., 1999). The mtDNA network was limited to the *cox1* sequences of *P. becki*. Samples of *P. variabilis* were included in the network of nuclear haplotypes. Three individuals contained a heterozygous site in the ITS-2 sequence data, and haplotypes for these individuals were therefore reconstructed using PHASE implemented in DnaSP (Stephens and Donnelly, 2003).

We used a relaxed molecular clock in BEAST v. 1.5.4 (Drummond and Rambaut, 2007) to estimate diversification times within the *P. becki* mtDNA phylogeography. Best-fit substitution models