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# Biogeography and colonization history of plethodontid salamanders from the Interior Highlands of eastern North America

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## ABSTRACT

**Aim** The Interior Highlands (Ouachita Mountains and Ozark Plateau) are major physiographical regions of eastern North America and harbour many endemic species. Despite their close proximity, the Ozarks and Ouachitas have different geological histories and relatively distinct species pools. Few studies have tested the biogeographical origins of this region's fauna, and most researchers have treated the Interior Highlands as a single unit. Here, we inferred the sources and timing of colonization of the Ozarks and the Ouachitas by analysing the biogeography of three genera of plethodontid salamanders (*Eurycea*, *Plethodon* and *Desmognathus*).

**Location** Eastern North America.

**Methods** We constructed a well-sampled, time-calibrated phylogeny for the family Plethodontidae using three mitochondrial and three nuclear genes in BEAST. Genetic data were primarily taken from GenBank, although we also produced 76 novel sequences. Using LAGRANGE, we reconstructed ancestral areas for North American plethodontids. We compared the frequency and timing of dispersal events between the Ozarks and Ouachitas to other putative sources such as the Eastern Highlands (Appalachian Mountains and associated limestone plateaus).

**Results** We inferred nine dispersal events from the Eastern Highlands to the Interior Highlands, and just two dispersal events between the geographically proximate Ozarks and Ouachitas. Following one dispersal in the Oligocene, other inter-highland dispersal events occurred in the Miocene and Pliocene, including two periods of near-synchronous movements.

**Main conclusions** Given the relatively limited faunal exchange between the Ozarks and Ouachitas, we conclude that either the river valley separating the Ozarks and Ouachitas is a more formidable barrier to plethodontid salamander dispersal than barriers separating the Interior Highlands from the Eastern Highlands, or ecological/community contingencies have limited dispersal within the Interior Highlands. In our study, geographical proximity of upland islands does not correspond with faunal similarity.

## Keywords

Amphibia, ancestral area reconstruction, Appalachian Mountains, comparative biogeography, dispersal, Mississippi River, Ouachita Mountains, Ozark Plateau, Plethodontidae, sky island

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## INTRODUCTION

Dispersal among habitat islands generally becomes more difficult with increasing isolation and decreasing island size

(MacArthur & Wilson, 1963, 1967). The ages and connectivity of 'sky island' habitats (Heald, 1951) have influenced the biodiversity of many organisms that are unable to disperse across lower elevations (reviewed by McCormack *et al.*,

2009). Therefore, highland regions in geographical proximity should have more similar faunas (or more frequent gene flow), while more distant regions should have less faunal exchange.

The highland regions of eastern North America are major centres of endemism and diversity for many mesic-adapted organisms, including amphibians (Duellman & Sweet, 1999), crayfishes (Crandall & Buhay, 2008) and freshwater fishes (Mayden, 1988). This is due to many factors, including their geological and climatic stability, complex topography and drainage systems, abundant springs (limestone geology), and relatively consistent precipitation (Cross *et al.*, 1986). The largest highland region in eastern North America is the Eastern Highlands, which includes the Appalachian Mountains and adjacent uplands.

Across the Mississippi River Valley to the west lie two smaller highland regions (Ouachita Mountains and Ozark Plateau), referred to collectively as the Interior Highlands. The Ozark Plateau is a dissected limestone uplift, and the Ouachita Mountains are a series of folded mountains, similar to the Appalachians but oriented on an east–west axis. Currently separated by the Arkansas River Valley, these adjacent highlands were formed by disparate geological processes, but both date to the Ouachita orogeny during the Pennsylvanian (Thornbury, 1965), and have since weathered extensively. Therefore, these highlands pre-date the origin of plethodontid salamanders by at least 200 Myr (Mueller, 2006; Zhang & Wake, 2009; Bonett *et al.*, 2014). Despite their geographical proximity (as close as ~38 km), the Ozarks and Ouachitas have distinct faunas, with over 160 and 48 endemic species respectively (Ouachita Ecoregional Assessment Team, The Nature Conservancy, 2003; Ozarks Ecoregional Assessment Team, The Nature Conservancy, 2003).

Few taxonomically broad studies concerning the origins of Interior Highland taxa have been conducted, although the fish fauna was the subject of several influential (but primarily descriptive) works nearly three decades ago (Mayden, 1985, 1987, 1988; Wiley & Mayden, 1985); Mayden (1985) also briefly discussed salamanders and crayfishes. Collectively, these authors concluded that the Appalachians, Ozarks and Ouachitas were contiguous until the Pleistocene, when they were fragmented by glaciation and associated processes. In contrast, Crandall & Templeton (1999) found evidence for multiple episodes of dispersal and vicariance among highland regions in the diverse crayfish genus *Orconectes*.

In one of the first discussions of Interior Highland biogeography, Dowling (1956) noted that salamanders had the most informative distributions for reconstructing past biogeographical events, due to their strict habitat requirements and low dispersal abilities. This is especially pronounced in lungless salamanders of the family Plethodontidae. Dowling concluded that there were four primary colonizing elements, including what he considered a ‘pre-glacial’ group from the Appalachians. As a rough metric to estimate timing of divergence, Dowling (1956) used taxonomic level (i.e. species versus subspecies), morphological and ecological differentiation,

and range continuity. However, in recent decades extensive DNA sequence data sets have been generated for eastern North American plethodontids, as well as methods for divergence time estimation and ancestral area reconstruction which can be used to better test these hypotheses.

Although the biogeography of eastern North American plethodontids has been analysed (Kozak *et al.*, 2009; Bonett *et al.*, 2014), previous authors treated the Ozarks and Ouachitas as a single biogeographical unit (Interior Highlands), despite their distinct faunas. To date, no study has tested the unique biogeographical origins of Ozark and Ouachita plethodontid salamanders. Here, we construct a time-calibrated phylogenetic hypothesis for the family Plethodontidae to infer the evolution of geographical ranges. From these reconstructions, we determine the geographical origins of Ozark and Ouachita plethodontids and compare the frequency of dispersal events among the three highland regions of eastern North America. Lastly, we use divergence time estimates to test for asynchrony of reconstructed dispersal events.

## MATERIALS AND METHODS

### Phylogenetic reconstruction

We sampled most recognized species of North American plethodontids, and several divergent lineages that potentially represent undescribed species, for a total of 230 terminal plethodontid taxa. We included two outgroup taxa, *Rhyacotriton variegatus* (Rhyacotritonidae) and *Amphiuma tridactylum* (Amphiumidae; see Appendix S1 in Supporting Information for species authorities). These represent families reconstructed as closely related to plethodontids in recent higher level molecular phylogenies of salamanders (Chippindale *et al.*, 2004; Wiens *et al.*, 2006; Roelants *et al.*, 2007; Zhang & Wake, 2009; Pyron & Wiens, 2011). We only sampled two to three representatives from each of the diverse Central and South American plethodontid genera (tribe Bolitoglossini) because these *in situ* radiations would not impact our North American ancestral area reconstructions, and omitting them increased computational efficiency. We referenced several published and unpublished intraspecific phylogenies to inform our taxon sampling, and included multiple representatives of species with particular relevance to our reconstruction, especially species with geographically divergent lineages distributed in multiple regions.

Our alignment included three mitochondrial genes: cytochrome *b* (*Cytb*), and NADH dehydrogenase subunits 2 (*ND2*) and 4 (*ND4*); and three nuclear genes: the recombination activating protein 1 gene (*Rag1*), pro-opiomelanocortin (*POMC*) and brain derived neurotrophic factor (*BDNF*). Most of the DNA sequences used were obtained from GenBank or other authors (716), but we also generated 74 novel sequences for this study following methods described previously (Shepard & Burbrink, 2008; Bonett *et al.*, 2014). Newly generated sequences were deposited on GenBank (see

Appendix S1 for sequence collection methods and GenBank accession numbers).

Alignments were constructed separately for each gene using CLUSTALW in MEGA 5.03 (Tamura *et al.*, 2007), and adjusted manually. Alignments were trimmed to regions containing at least 10 overlapping sequences. The alignment matrix totalled 5520 nucleotide positions, was 56.6% complete (including gaps), and contained the following numbers of sequences per gene: *Cytb* (192 taxa), *ND2* (129 taxa), *ND4* (192 taxa), *Rag1* (205 taxa), *POMC* (73 taxa) and *BDNF* (39 taxa). There was a significant amount of missing data, especially for *POMC* and *BDNF*; however, these absences generally do not appear to have a major impact on phylogenetic reconstructions (Wiens, 2006; Wiens & Morrill, 2011) or divergence dates (Zheng & Wiens, 2015), and incompletely sampled genes can still improve phylogenetic resolution (Jiang *et al.*, 2014). The sequences were distributed across taxa (most genera had at least one sequence for all genes) and the less well-sampled genes contained phylogenetic signal that helped to resolve deeper nodes. Phylogenetic analyses excluding *POMC* and *BDNF* failed to approach stationarity after twice as many generations and although the resulting trees recovered very similar relationships among terminal taxa, deeper nodes were poorly resolved (not shown). Therefore, we concluded the potential negative effects of missing data on topology, support values, and branch-length estimation (Lemmon *et al.*, 2009) were preferable to the loss of resolution that occurred when these genes were excluded. Taxa without data for a given gene were included in the alignments as missing data for every nucleotide position. Our sampling strategy was designed to maximize taxon sampling for ancestral area reconstruction while minimizing the creation of chimeric taxa (samples consisting of sequences assembled from multiple individuals of a species), which are undesirable because different genes in chimeric taxa may yield discordant gene trees (Maddison, 1997). Our phylogenetic estimate is highly congruent topologically with prior studies (see Results), indicating our sampling strategy had a negligible influence on our results.

We used jMODELTEST 2.1.4 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) to determine the best substitution model for each gene. Searches among substitution models were limited to models available in the BEAUTI configurator, which was used to generate the .xml file for BEAST 1.7.4 (Drummond *et al.*, 2012). To reduce computation time, we constrained the monophyly of the family Plethodontidae and the genera *Aneides*, *Dendrotriton*, *Desmognathus*, *Eurycea* and *Plethodon*, all of which have been supported by previous studies (Chippindale *et al.*, 2004; Vieites *et al.*, 2007, 2011; Kozak *et al.*, 2009).

To examine dispersal timing, we constrained the crown-group age of Plethodontidae to  $73 \pm 6$  Ma (following Bonett *et al.*, 2014; see references therein), which provided median estimates for the ages of nodes reconstructed to represent dispersal events (see Ancestral area reconstruction, below), as well as upper and lower confidence limits on age estimates

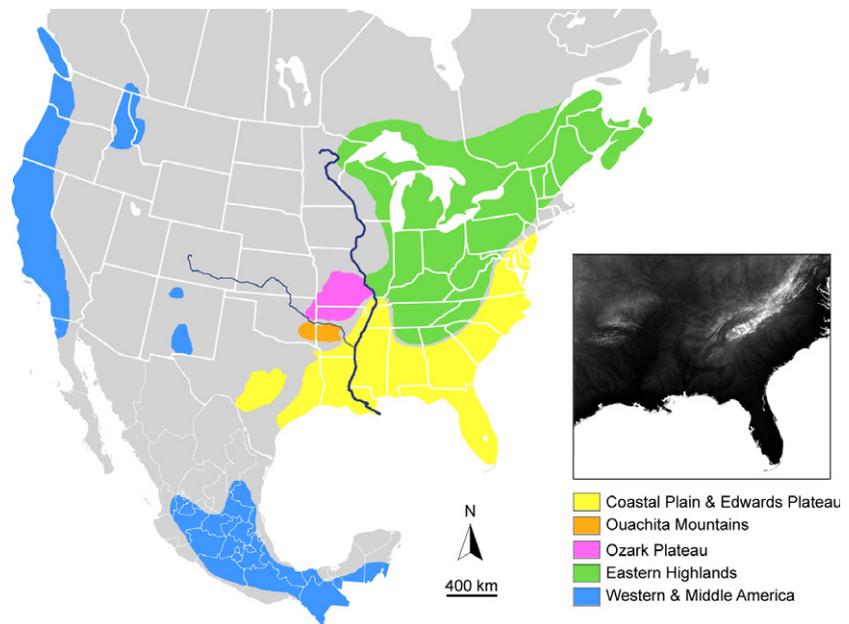
[95% highest posterior density (HPD)]. Date estimates for crown-group plethodontids range from 99 to 65 Ma (Muller, 2006; Roelants *et al.*, 2007; Vieites *et al.*, 2007; Zhang & Wake, 2009; Bonett *et al.*, 2014), but the most common estimates are in the range of 65–85 Ma. Our root age prior spanned most of that timeframe. Although absolute divergence dates may be somewhat inaccurate, we were primarily interested in whether relative dates overlapped, and thus whether any dispersal events may have been synchronous (i.e. overlapping 95% HPDs).

We performed five independent MCMC searches of  $1.0 \times 10^8$  generations in BEAST using the CIPRES Science Gateway (Miller *et al.*, 2010), logging trees and parameters every 10,000 generations. We applied unlinked substitution models and clock models, linked tree models (functionally concatenated), a Yule process speciation prior, and uncorrelated lognormal relaxed clock rate priors (mean = 0, SD = 1). We used LOGCOMBINER 1.7.4 to pool log and tree files from all five runs, conservatively discarding the first 25% of generations per run as burn-in and resampling every tenth logged generation to reduce file sizes. We used TRACER 1.5 (Drummond & Rambaut, 2007) to confirm that independent runs had converged and effective sample size values were > 200 for all parameters in the combined log file. We calculated the maximum clade credibility (MCC) tree using TREEANNOTATOR 1.7.4 with the combined posterior distribution of trees.

### Ancestral area reconstruction

After pruning two outgroups (*Amphiuma* and *Rhyacotriton*) and three Eurasian plethodontids (*Karsenia koreana*, *Hydromantes genei* and *Hydromantes italicus*) from the BEAST chronogram, we reconstructed ancestral areas for North American Plethodontidae using the dispersal-extinction-cladogenesis (DEC) model in LAGRANGE v. 20120508 (Ree & Smith, 2008). Taxa were coded as occurring in one or more of five regions (Fig. 1): (A) Ouachita Mountains; (B) Ozark Plateau; (C) Coastal Plain plus Edwards Plateau (combined given that Edwards Plateau taxa are nested within Coastal Plain clades); (D) Eastern Highlands (including the Appalachian Mountains and northward to the Great Lakes; species dispersal from the Eastern Highlands north to the Great Lakes occurred recently (< 21 ka; Highton & Webster, 1976); and (E) Western North America (WNA) plus Central and South America (because the clade in Central and South America is sister to a clade in western North America). Species were coded as inhabiting an area if they occurred there, regardless of their extent of occurrence in that region, and allowed to inhabit up to four regions (a realistic assumption based on the distributions of extant taxa). Transition probabilities were equally weighted, and direct dispersal between WNA and the Eastern Highlands was disallowed, given that salamanders would have to disperse from east to west via one of the intervening regions (Coastal Plain, Ozarks, or Ouachitas). An additional LAGRANGE analysis without this

**Figure 1** Approximate distribution of North American plethodontid salamanders with colours corresponding to five areas used for ancestral area reconstruction coding: Orange (Ouachita Mountains), Pink (Ozark Plateau), Yellow (Coastal Plain plus Edwards Plateau), Green (Eastern Highlands, including the Appalachian Mountains northward to the Great Lakes) and Blue (western North America plus Middle and South America). The courses of the Mississippi and Arkansas rivers are represented by thick and thin dark blue lines respectively. Inset relief map of eastern North America shows the geographical separation among the Eastern Highlands, Ouachitas and Ozarks.



dispersal constraint produced nearly identical results, with the exception of a few basal nodes (see Appendix S2 for this alternative reconstruction). We considered each event where a descendant node was reconstructed to inhabit a region uninhabited by its parent node to be a dispersal event.

## RESULTS

### Phylogeny

Our phylogeny showed strong support ( $\geq 95\%$  Bayesian posterior probability) for many recognized clades and all genera (see Appendix S3 for complete BEAST phylogram with support values), and its topology agrees well with recent phylogenetic hypotheses for the Plethodontidae (Chippindale *et al.*, 2004; Mueller *et al.*, 2004; Kozak *et al.*, 2009; Pyron & Wiens, 2011; Vieites *et al.*, 2011; Bonett *et al.*, 2014). *Hemidactylium* was recovered as sister to Bolitoglossini, in agreement with prior studies (Mueller *et al.*, 2004; Vieites *et al.*, 2011), although some studies have recovered *Hemidactylium* as sister to Bolitoglossini plus Spelerpini (Kozak *et al.*, 2009; Pyron & Wiens, 2011). The placement of *Hemidactylium* would only affect ancestral area reconstructions for the deepest nodes in the tree, whereas we are primarily focused on events that occurred at more shallow nodes (e.g. within genera). We also note that *Plethodon sequoyah* was nested within *P. albagula*, a relationship which has been reported previously (Kozak *et al.*, 2006; Wiens *et al.*, 2006).

### Historical biogeography

Our LAGRANGE analysis indicated that the most recent common ancestor (MRCA) of Plethodontidae inhabited western North America (WNA), or WNA plus the Eastern Highlands and Coastal Plain (Fig. 2). Likewise, the MRCA of the genus

*Plethodon* also likely inhabited WNA, while the MRCA of eastern *Plethodon* was reconstructed to have inhabited either the Coastal Plain (support 0.268; Fig. 2) or the Ouachitas (support 0.255). The MRCAs of the tribe Spelerpini and genus *Desmognathus* inhabited the Eastern Highlands (support 0.44 and 0.84 respectively).

Within eastern North America, our analysis indicated frequent movement from east to west, with nine reconstructed dispersal events from the Eastern Highlands and Coastal Plain to the Interior Highlands (Ozarks and Ouachitas). In contrast, movement between the two Interior Highland regions has been infrequent, with only two reconstructed dispersal events from the Ouachitas to the Ozarks (and none from Ozarks to Ouachitas). However, we note that two dispersal events from the Eastern Highlands were reconstructed as simultaneous colonizations of the Ozarks and Ouachitas; alternatively, these taxa may have colonized one Interior Highland region first and subsequently dispersed to the other, potentially increasing the number of dispersal events between the Ouachitas and Ozarks to four. Our alternative LAGRANGE analysis allowing direct transitions between WNA and the Eastern Highlands (see Appendix S2) indicated this may have occurred in the Interior Highlands *Eurycea* clade. We inferred no dispersal events from the Interior Highlands to the Eastern Highlands within eastern *Plethodon*, although *Plethodon serratus* likely dispersed south from the Ouachitas into the Coastal Plain.

The ancestor of Interior Highlands *Eurycea* was reconstructed to have colonized the Ozarks and Ouachitas simultaneously (support 0.230) from the Eastern Highlands, approximately 28.9 Ma (31.3–26.5 Ma; Fig. 3). Support was lower for the ancestor initially colonizing only the Ozarks (0.211) or Ouachitas (0.153). Subsequent vicariance and *in situ* speciation produced two Ozark endemic species (*E. tynerensis* and *E. spelaea*) and two Ouachita endemics

(*E. multiplicata* and *E. subfluvicola*). A population of *E. multiplicata* also dispersed from the Ouachitas to the Ozarks more recently (3.3 Ma, 3.6–3.0 Ma). *Eurycea lucifuga* colonized the Ozarks from the Eastern Highlands in the Pliocene (4.0 Ma, 4.3–3.7 Ma), although this reconstruction was weakly supported (0.326) over colonization of the Eastern Highlands from the Ozarks (0.321). We feel the former reconstruction is more likely given the greater genetic diversity of *E. lucifuga* in the Eastern Highlands (Timpe, 2009). The ancestor of the ‘long-tailed’ clade (*E. longicauda* and *E. guttolineata*) expanded from the Coastal Plain into the Eastern Highlands and Ozarks (10.1 Ma, 10.9–9.3 Ma). Although there was low support for the reconstruction at the parent node of the ‘long-tailed’ clade plus *E. lucifuga* (0.326; Fig. 2), alternative reconstructions also supported a widespread ancestor for the ‘long-tailed’ clade. Subsequent vicariant events resulted in *E. longicauda* in the Eastern Highlands and *E. l. melanopleura* in the Ozarks.

The Ouachita endemic *Desmognathus brimleyorum* was phylogenetically nested within a large clade of primarily Eastern Highland species. Therefore, we are confident that the ancestor of *D. brimleyorum* colonized the Ouachitas from the Eastern Highlands (support 0.624), although a different topology might impact the age of the node and time of dispersal (16.0 Ma, 17.4–14.7 Ma).

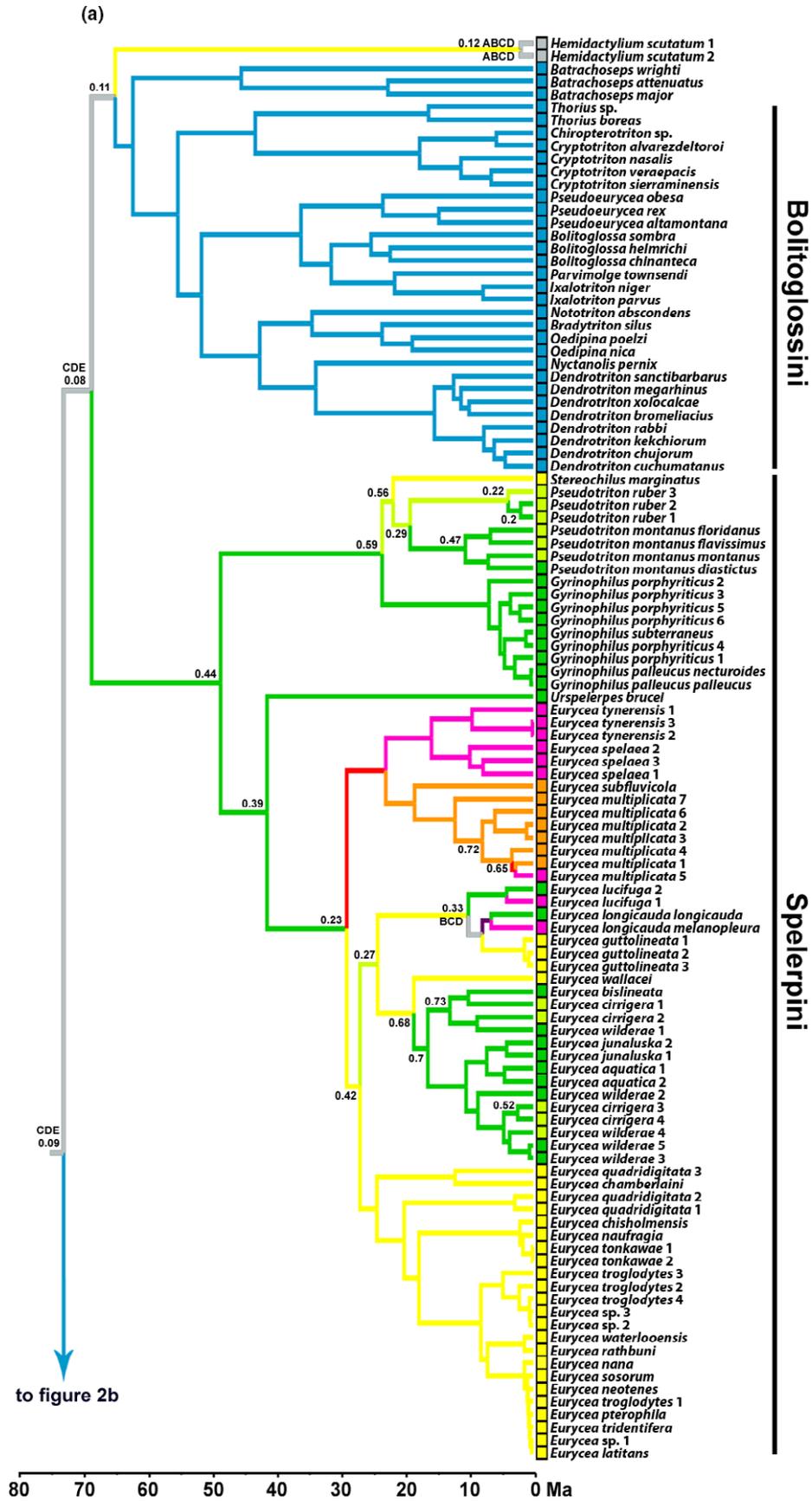
Salamanders of the genus *Plethodon* have experienced many more dispersal/extinction events than *Eurycea* and *Desmognathus*. *Plethodon serratus* was reconstructed to have colonized both Interior Highland areas simultaneously (support 0.524) from the Eastern Highlands (support 0.289; 4.3 Ma, 4.7–4.0 Ma), and subsequently dispersed into the Coastal Plain from the Ouachitas. *Plethodon angusticlavius* colonized the Ozarks from the Eastern Highlands (8.7 Ma, 9.4–8.0 Ma). According to the most probable reconstruction, the ‘Ouachita slimy’ clade (*P. ouachitae*, *P. caddoensis*, and *P. fourchensis*) originated from vicariance of a wide-ranging *Plethodon* between the Eastern Highlands and the Ouachitas, followed by vicariant speciation among different Ouachita mountaintops (Shepard & Burbrink, 2008, 2009, 2011). This wide-ranging *Plethodon* ancestor had previously colonized the Ouachitas from the Eastern Highlands in the Miocene (12.2 Ma, 13.2–11.2 Ma). However, this reconstruction was weakly supported (0.505) over dispersal to the Ouachitas from the Eastern Highlands by the MRCA of the ‘Ouachita slimy’ clade (support 0.480; 11.5 Ma, 12.4–10.5 Ma). The common ancestor of *P. mississippi* and *P. kiamichi* expanded its range from the Eastern Highlands into the Coastal Plain and Ouachitas (support 0.341) at the Miocene-Pliocene boundary (5.4 Ma, 5.8–4.9 Ma); there was also marginal

support for the ancestor only inhabiting the Ouachitas and Eastern Highlands (0.257) or the Coastal Plain and Eastern Highlands (0.228). The populations in the Ouachitas were later isolated, giving rise to *P. kiamichi*. Finally, *P. albagula* colonized the Ouachitas from the Coastal Plain/Edwards Plateau (3.5 Ma, 3.8–3.2 Ma). Our reconstructions indicated a population-level range expansion from the Ouachitas to the Ozarks (support 0.749; 3.0 Ma, 3.3–2.8 Ma), which captured some of the ancestral variation of the parent population before vicariance between the Ozarks and Ouachitas. Alternatively, there was weak support (0.242) for two independent dispersal events by *P. albagula* from the Ouachitas to the Ozarks. Finer scale phylogeographical sampling is necessary to distinguish between these two scenarios, and to assess the validity of *P. sequoyah*.

The first colonization of the Interior Highlands, by the ancestor of Interior Highlands *Eurycea* in the Oligocene (Fig. 3), far preceded other reconstructed dispersal events. The next colonization, by the ancestor of *Desmognathus brimleyorum*, occurred in the Miocene several million years before three roughly synchronous (13.2–8 Ma) dispersal events by ancestors of the ‘Ouachita slimy’ clade of *Plethodon*, the ‘long-tailed’ *Eurycea* clade, and *P. angusticlavius*. The common ancestor of *P. mississippi* and *P. kiamichi* dispersed out of the Eastern Highlands around the Miocene-Pliocene boundary. The remaining five dispersal events (*P. serratus*, *E. lucifuga*, *P. albagula* to the Ouachitas, *E. multiplicata* and *P. albagula* to the Ozarks) had overlapping date estimates during the Pliocene (4.7–2.8 Ma).

Our alternative reconstruction, which allowed direct transitions between WNA and the Eastern Highlands, gave very similar results (see Appendix S2). Most of the differences were expected; for example, several branches formerly reconstructed as Coastal Plain (because they were transitions from WNA to Eastern Highlands) were supported as direct transitions to the Eastern Highlands (MRCAs of *Hemidactylum*, *Aneides aeneus*, *Desmognathus* plus *Phaeognathus* and eastern *Plethodon*). However, there were some other differences relevant to the Interior Highlands. The MRCA of the Interior Highlands *Eurycea* clade was reconstructed to have colonized the Ozarks only, followed by colonization of the Ouachitas by the MRCA of *E. multiplicata* and *E. subfluvicola*. The MRCA of the ‘Ouachita slimy’ *Plethodon* was reconstructed to have dispersed from the Eastern Highlands, rather than resulting from vicariance of a widespread ancestral *Plethodon*. Finally, the MRCA of *Plethodon albagula* was reconstructed as inhabiting both the Ouachitas and Coastal Plain, with subsequent vicariance between the two areas.

**Figure 2** BEAST chronogram of North American plethodontid salamanders, presented in two parts labelled (a and b), with five colours indicating highest probability ancestral area reconstruction in LAGRANGE: (A) **orange** (Ouachita Mountains), (B) **pink** (Ozark Plateau), (C) **yellow** (Coastal Plain plus Edwards Plateau), (D) **green** (Eastern Highlands, including the Appalachian mountains northward to the Great Lakes), and (E) **blue** (western North America plus Central and South America). Other colours represent area combinations: **grey** (3+ areas, labelled), **red** (A+B), **brown** (A+D), **purple** (B+D), **light green** (C+D) and **dark teal** (C+E). LAGRANGE support values < 75% labelled; all unlabelled nodes received at least 75% support for ancestral area reconstruction.



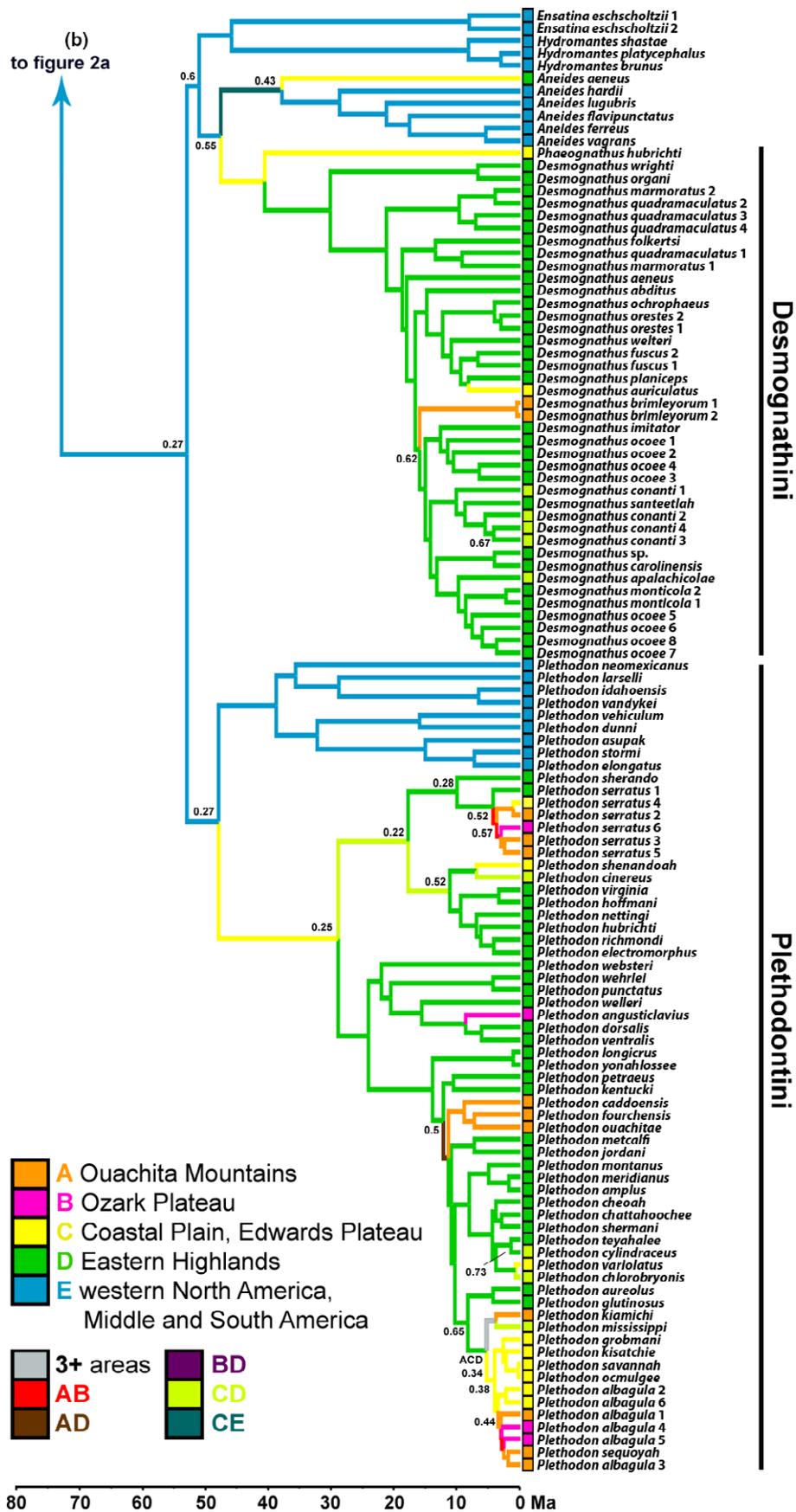
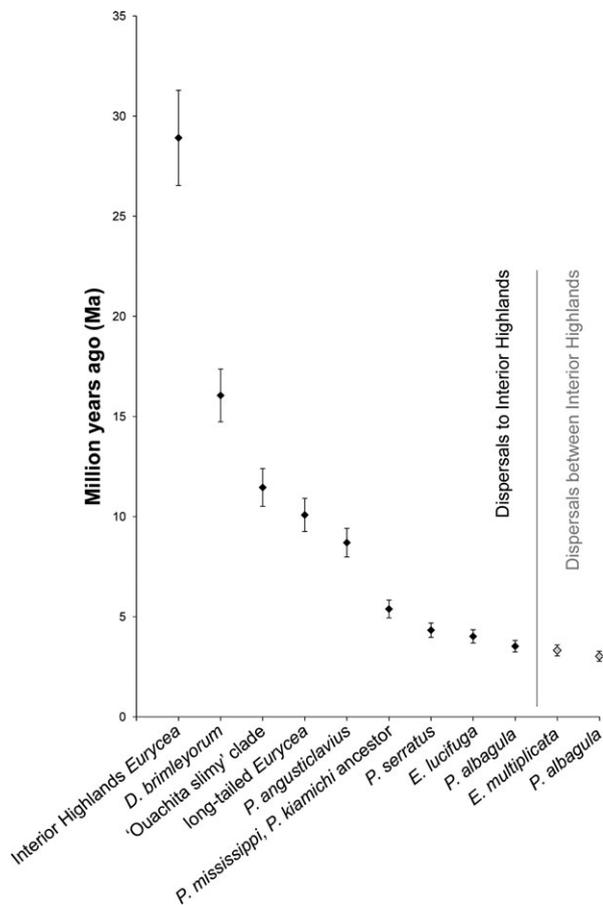


Figure 2 Continued.



**Figure 3** Estimated node ages (from BEAST chronogram) of dispersal events (according to LAGRANGE reconstruction) by plethodontid salamanders to one or both of the Interior Highland regions (Ozark Plateau or Ouachita Mountains). Error bars represent upper and lower age estimates (95% Highest Posterior Density) based on a crown-group age of  $73 \pm 6$  Ma for Plethodontidae (following Bonett *et al.*, 2014). Nodes are arranged from oldest to youngest. Black diamonds represent dispersal events from the Eastern Highlands or Coastal Plain to the Interior Highlands, and grey diamonds represent dispersal events between the Ozarks and Ouachitas.

## DISCUSSION

### Colonization of the Interior Highlands

The Eastern Highlands are the largest upland region in eastern North America, and historically contained the largest pool of species for potential colonizations of the Ozarks and Ouachitas (Kozak & Wiens, 2012). Our biogeographical reconstructions supported the Eastern Highlands as the origin for Interior Highlands *Desmognathus*, *Eurycea*, and *Plethodon* (Fig. 2). The Eastern Highlands are also thought to be the source for other Interior Highland fauna, including *Nothonotus* darters (Near & Keck, 2005), *Orconectes* crayfishes (Crandall & Templeton, 1999; and references therein), and numerous insects (Allen, 1990). Population and geographical range sizes of species may also influence the

likelihood of colonization with abundant and wide-ranging species being more likely colonizers. Little is known about population sizes of most plethodontids and species distributions are highly variable in size. Genetic data and distribution models for some extant species indicate that population sizes and geographical ranges have expanded and contracted historically (Shepard & Burbrink, 2008, 2009, 2011; Pelletier *et al.*, 2014). However, these traits would be difficult or impossible to determine for the ancestral lineages that were inferred to have colonized new regions.

Despite the ancient age of the Interior Highlands (> 300 Ma) and plethodontids (~73 Ma), only one extant, endemic lineage of plethodontids arrived during the Paleogene (Interior Highlands *Eurycea*; Fig. 3). This lineage subsequently diversified *in situ* within both the Ozarks and the Ouachitas, including considerable diversification in life history and morphology (Bonett & Chippindale, 2004). The ancestor of the 'Ouachita slimy' clade (*Plethodon ouachitae* group) colonized the Ouachitas in the Miocene and diversified into several ecologically similar, parapatric and allopatric species (Shepard & Burbrink, 2008, 2009, 2011). Other Miocene colonizers of the Interior Highlands showed no evidence of *in situ* diversification (ancestors of *Desmognathus brimleyorum*, *Eurycea longicauda melanopleura*, and *P. angusticlavius*). The remaining plethodontid lineages colonized the Interior Highlands more recently, in the Pliocene. While Dowling (1956) concluded that the troglobitic 'Typhlotriton' (*Eurycea spelaea*) was the only Interior Highland faunal element older than 2.5 Ma, we found that all plethodontid colonizations were considerably older than the Pleistocene. Both *Nothonotus* darters and amblyopsid cavefishes probably dispersed to the Interior Highlands in the Miocene as well as more recently, in the late Pliocene/early Pleistocene (Near & Keck, 2005; Niemiller *et al.*, 2013).

One possible explanation for the apparent rarity of 'old' dispersal events (e.g. Oligocene) was the smaller pool of potential colonizers at that time. According to our reconstructions, only six plethodontid lineages were present in the Eastern Highlands when the MRCA of Interior Highlands *Eurycea* dispersed (31.3–26.5 Ma; Fig. 3). Other lineages may have existed, but gone extinct without leaving extant descendants. However, by the time the ancestor of *D. brimleyorum* dispersed to the Interior Highlands in the Miocene there were already nearly 20 plethodontid lineages present in the Eastern Highlands, with over 70 by the end of the Miocene (Fig. 2). This growing species pool may have provided more potential species for colonization of the Interior Highlands. In contrast to the frequency of colonizations from the Eastern to the Interior Highlands, there were few dispersal events between the Ouachitas and Ozarks.

### Infrequent dispersal within the Interior Highlands

Dispersal among habitat islands should be more common with decreasing geographical separation (MacArthur & Wilson, 1963, 1967); therefore, we expected upland regions in close

proximity to have more frequent faunal exchange. Mayden (1987) concluded that the fish faunas of the Ozarks and Ouachitas were more similar to one another than to the Eastern Highlands; however, we found the opposite pattern in plethodontid salamanders. Few plethodontids have dispersed between the Ozarks and Ouachitas despite their geographical proximity (as close as 38 km), while the more distant Eastern Highlands have been the source for multiple colonizations of the Interior Highlands. Our findings are consistent with a biogeographical reconstruction for *Nothonotus* darters indicating rare dispersal among highland regions of eastern North America (Near & Keck, 2005), a non-sister relationship between the Ozarks and Ouachitas in the *Notropis rubellus* species group (Berendzen *et al.*, 2008), and a recent study showing that parts of the Ozark and Ouachita fish faunas are distantly related due to a long period of drainage isolation (Hoagstrom *et al.*, 2013).

One possible explanation for the rarity of plethodontid dispersal between the Ozarks and Ouachitas despite their geographical proximity is that they were/are separated by a relatively impermeable barrier. The Ozarks and Ouachitas were formed by disparate geological processes in the Pennsylvanian (Thornbury, 1965), and have been disjunct highlands since their formation. Although the lower Arkansas River may not have followed its present course bisecting the Interior Highlands until the Pleistocene (Quinn, 1958; Galloway *et al.*, 2011), the primary drainages of the Interior Highlands have probably not changed appreciably since the Pennsylvanian (Pfleiger, 1971). The Ozarks and Ouachitas have always been separated by lowland habitat (the Arkoma Basin; Carlton & Cox, 1990), which would limit dispersal in many plethodontid salamanders. However, due to the narrow width of this putative barrier, other factors may have contributed to the infrequency of dispersal.

The size and course of the Mississippi River have been fluid over geological time, and it only reached its current northern extent in the middle Miocene (Galloway *et al.*, 2011), approximately ~16–12 Ma. However, the Eastern and Interior Highlands have been separated south of the present-day confluence of the Mississippi and Ohio Rivers by a lowland barrier (the Mississippi Embayment) since the end of the Cretaceous (~65 Ma; Cushing *et al.*, 1964). The Mississippi Embayment and lower Mississippi River have limited dispersal by many terrestrial organisms (Soltis *et al.*, 2006; Lemmon *et al.*, 2007; Pyron & Burbrink, 2010). Therefore, the relative frequency of plethodontid dispersal across this barrier is surprising, but several possible explanations exist.

Ecological contingencies may have influenced dispersal; the permeability of intervening lowland habitats may not be as important as the similarity (or disparity) of habitats in these adjacent uplands. The different geologies of the Ozarks and Ouachitas have consequences for the types of habitats available to plethodontid salamanders. The Ozarks are characterized by lower relief upland streams fed by abundant springs, due to the karst topography. This habitat is amenable to species dependent on consistent water sources

and/or subterranean environments, such as *Eurycea* (Bonett & Chippindale, 2004; Bonett *et al.*, 2014).

In contrast, the Ouachitas are composed of several narrow, east–west trending mountain ranges with short, steep drainages, scattered across low-relief lowlands. Surface stream flow is more intermittent and the Ouachitas support only two *Eurycea*. There are at least seven species of *Plethodon* in the Ouachitas, compared to three in the Ozarks. These direct-developing, terrestrial salamanders are less dependent on standing water sources compared to species with aquatic larvae, but still require mesic woodland microhabitats. The higher frequency of inter-highland movements by *Plethodon* may be due to their species diversity and abundance (larger pool of potential colonizers), as well as direct development and increased terrestriality (and thus improved dispersal abilities) relative to other plethodontids. For example, more than 75% of the current range of the widely distributed *Plethodon cinereus* was glaciated and uninhabitable just 21,000 years ago (Highton & Webster, 1976), indicating a great capacity for dispersal in some species.

Although habitats of the Ozarks and Ouachitas are different, habitats in the Ozarks and plateaus of the Eastern Highlands (Cumberland and Interior Low Plateaus) are similar, with karst topography and abundant springs. Regardless of whether formerly continuous northern uplands linked the Ozarks and eastern plateaus prior to the Pleistocene as posited by Mayden (1985), this karst topography is continuous across southern Illinois and Indiana, linking the Ozarks and eastern plateaus geologically. Several plethodontids are associated with karst topography in both the Ozarks and Eastern Highlands, particularly *Eurycea lucifuga* and *E. longicauda*. The distributions of karst-associated cave crayfishes (genus *Orconectes*; Crandall & Templeton, 1999) and fishes such as *Typhlichthys subterraneus* (Niemiller *et al.*, 2012), *Forbesichthys agassizi* (Page & Burr, 2011), and *Cottus carolinae* (which exhibits recent gene flow via southern Illinois; Strange & Burr, 1997) spanning the Mississippi River also seem to support habitat continuity from the eastern plateaus to the Ozarks. Therefore, habitat similarity may help explain the frequency of dispersal and successful establishment from the Eastern Highlands to the Ozarks. The Mississippi River's historically smaller size and lower northern extent may have also facilitated dispersal.

Finally, the smaller number of plethodontid species in the Interior Highlands has limited the pool of potential dispersers between the Ozarks and Ouachitas, and from the Interior Highlands to the Eastern Highlands. Our reconstructions indicated that no Interior Highland plethodontids have recolonized the Eastern Highlands. However, some fishes (Martin & Bonett, *unpublished*), crayfishes (Crandall & Templeton, 1999), and cryptobranchid salamanders (Sabatino & Routman, 2009) have probably dispersed from the Ozarks to the Eastern Highlands. The permeability of lowland barriers, contingencies of habitat similarity or disparity and varying sizes of species pools for potential colonization are not mutually exclusive hypotheses, and all may have contributed

to the observed patterns of plethodontid dispersal and colonization. We note that dispersal may have been more frequent, but its signature has been erased by extinction; upland habitats offer poor conditions for fossil formation and salamanders' fragile bones are rarely preserved.

### Additional evidence for dispersal events

Because our phylogenetic analysis did not include sequences of cytochrome oxidase 1 (*COI*), the only geographically well-sampled marker for the widespread four-toed salamander (*Hemidactylium scutatum*), we were unable to reconstruct its phylogeographical history in concert with other plethodontids. Herman (2009) concluded that the ancestors of the Ozark and Ouachita populations of *H. scutatum* independently dispersed from the Eastern Highlands, 0.2 Ma and 0.4 Ma respectively. If this reconstruction is accurate, it would add two more dispersal events from the Eastern Highlands to the Interior Highlands, and would represent the only Pleistocene plethodontid dispersal.

Historical collection records exist for *Desmognathus 'brimleyorum'* from north of the Arkansas River in Arkansas [e.g. Crawford (KU 4601–4604), Pope, Pulaski and Faulkner counties; K. Roberts, pers. comm.]. Most of these localities are located in the Arkansas River Valley just south of the Ozark Plateau, but potentially represent one or more dispersal events from the Ouachitas northward across the Arkansas River. There were also formerly populations of *Desmognathus conanti* on Crowley's Ridge in north-eastern Arkansas (a narrow highland ridge that emerges from the Mississippi River floodplain west of the river; Means, 1974; Trauth *et al.*, 2004). These populations represent an additional east–west dispersal, potentially across the Mississippi River. Alternatively, these populations may have become isolated on the west side of the Mississippi River when the river changed from its former course west of Crowley's Ridge to its current course to the east (Blum *et al.*, 2000). Unfortunately, *Desmognathus* have not been found on Crowley's Ridge since before 1990 (Trauth *et al.*, 2004), and we cannot determine the relationships or timing of colonization of these populations without genetic samples.

### KEY CONCLUSIONS

Although the Ozark and Ouachita uplands are geographically proximate, they have had little faunal exchange, due to intervening barriers, differing habitats and/or smaller species pools. Instead, most plethodontids dispersed from the Eastern to the Interior Highlands. Furthermore, most of these dispersal events occurred during the Miocene and Pliocene, although better sampling and additional data are needed to resolve recent dispersal events (e.g. *Hemidactylium scutatum*, *Plethodon albagula*). Taken together, our findings indicate substantial but asymmetrical faunal exchange between the Eastern and Interior Highlands, and support a growing consensus that many Interior Highland species have pre-

Pleistocene origins (Mayden, 1988; Strange & Burr, 1997; Near & Keck, 2005; Berendzen *et al.*, 2008).

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## SUPPORTING INFORMATION

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

**Appendix S1** GenBank accession numbers, ancestral area coding, primer information, and species authorities.

**Appendix S2** Alternative ancestral area reconstruction, with transitions between the Eastern Highlands and western North America allowed.

**Appendix S3** Unpruned phylogram with BEAST support values < 95% labelled.

## DATA ACCESSIBILITY

Genetic data used in this study can be found on GenBank; see Appendix S1 for a list of accession numbers.

## BIOSKETCH

**Samuel D. Martin** primarily studied biogeography and macroevolution in minnows for his doctoral research. However, his academic pursuits began with and still include amphibian ecology and evolution, which is why his co-authors haven't ostracized him.

Author contributions: R.M.B. and S.D.M. conceived the study; all authors contributed sequence data and advised taxon sampling; S.D.M. analysed the data and prepared the manuscript draft; S.D.M., R.M.B. and D.B.S. interpreted the results; and all authors read and approved the final manuscript.

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