



EVOLUTION OF PAEDOMORPHOSIS IN PLETHODONTID SALAMANDERS: ECOLOGICAL CORRELATES AND RE-EVOLUTION OF METAMORPHOSIS

Ronald M. Bonett,^{1,2} Michael A. Steffen,¹ Shea M. Lambert,³ John J. Wiens,³ and Paul T. Chippindale⁴

¹Department of Biological Science, University of Tulsa, Tulsa, Oklahoma 74104

²E-mail: ron-bonett@utulsa.edu

³Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

⁴Department of Biology, University of Texas Arlington, Arlington, Texas 76019

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Life-history modes can profoundly impact the biology of a species, and a classic example is the dichotomy between metamorphic (biphasic) and paedomorphic (permanently aquatic) life-history strategies in salamanders. However, despite centuries of research on this system, several basic questions about the evolution of paedomorphosis in salamanders have not been addressed. Here, we use a nearly comprehensive, time-calibrated phylogeny of spelerpine plethodontids to reconstruct the evolution of paedomorphosis and to test if paedomorphosis is (1) reversible; (2) associated with living in caves; (3) associated with relatively dry climatic conditions on the surface; and (4) correlated with limited range size and geographic dispersal. We find that paedomorphosis arose multiple times in spelerpines. We also find evidence for re-evolution of metamorphosis after several million years of paedomorphosis in a lineage of *Eurycea* from the Edwards Plateau region of Texas. We also show for the first time using phylogenetic comparative methods that paedomorphosis is highly correlated with cave-dwelling, arid surface environments, and small geographic range sizes, providing insights into both the causes and consequences of this major life history transition.

KEY WORDS: Amphibians, biogeography, climate, development, life history, phylogeny.

Life-history transitions can have many consequences for the biology of a lineage (e.g., Stearns 1992; Moran 1994). An especially dramatic example is the phenomenon of larval paedomorphosis in salamanders, wherein adults forego metamorphosis and maintain an aquatic larval morphology and lifestyle (e.g., Gould 1977). Paedomorphosis has evolved many times throughout the history of salamanders. These shifts range from ancient transitions in strictly paedomorphic families of Mesozoic age (e.g., amphiumids, cryptobranchids, proteids, sirenids; Wiens et al. 2005; Bonett et al. 2013) to more recent transitions among closely related species in the same genus (e.g., *Ambystoma* and *Eurycea*; Chippindale et al. 2000; Bonett and Chippindale 2004; Weisrock et al. 2006). Given the many origins of paedomorphosis, salamanders offer an

important model to study the ecological correlates and macroevolutionary consequences of life-history transitions.

The evolution of paedomorphosis in salamanders has been of great interest to biologists for centuries (early literature reviewed in Gould 1977). A wealth of research has focused on intraspecific trade-offs associated with paedomorphosis and metamorphosis on “ecological time scales” (Wilbur and Collins 1973; White-man 1994; Denöel et al. 2005; Bonett and Chippindale 2006). However, many fundamental questions about the evolution of paedomorphosis in salamanders have not been addressed among species in a phylogenetic context. These include: is paedomorphosis reversible? In other words, once metamorphosis is lost, can it be regained? What are the ecological conditions that drive

the evolution of paedomorphosis? For example, theory suggests that paedomorphosis is most likely to evolve when environmental conditions experienced by adults are inhospitable relative to conditions experienced by larvae (e.g., Wilbur and Collins 1973). This might include environments that are too dry for terrestrial adults, or subterranean aquifers where terrestrial microhabitats are limited or resource poor. Some evidence is suggestive of these patterns. It has been noted that many paedomorphic plethodontids occur in caves (e.g., Wake 1966; Ryan and Bruce 2000) and many paedomorphic ambystomatids and plethodontids seem to occur in the drier portions of the ranges of these families (e.g., Sprules 1974; Sweet 1977). However, these patterns have yet to be explicitly tested, and a statistical phylogenetic framework is especially important for this. Many questions also remain about the biological consequences of paedomorphosis. For example, do paedomorphic species have smaller range sizes than their metamorphosing relatives, given that their ability to disperse overland may be greatly reduced, if not completely lost? Here, we address these questions in a statistical phylogenetic context in plethodontid salamanders, specifically the subfamily Spelerpinae (sensu Chippindale et al. 2004; Pyron and Wiens 2011).

In many ways, spelerpines are an ideal system in which to address these questions, compared to most other salamanders. Spelerpinae contains five genera and 35 described species (AmphibiaWeb 2013). Many species groups have been the subject of in-depth systematic studies, making the distributional limits of lineages relatively well known (e.g., Chippindale et al. 2000; Kozak et al. 2006; Timpe et al. 2009; Emel and Bonett 2011). Paedomorphosis appears to have evolved in several clades of spelerpines (Wake 1966; Ryan and Bruce 2000; Chippindale et al. 2000; Bonett and Chippindale 2004; Niemiller et al. 2008), which provides independent replication for phylogenetically based statistical analyses. In addition, a nominal species that appears to be deeply nested among paedomorphic lineages has some metamorphic populations (*Eurycea troglodytes*; Sweet 1977; Chippindale et al. 2000), raising the possibility that metamorphosis may have re-evolved in this lineage. The apparently recent origins of paedomorphosis in spelerpines also facilitate testing the ecological correlates of these transitions. These contrast with the seemingly ancient origins of paedomorphosis in many other families (e.g., amphiumids, cryptobranchids, proteids, sirenids; e.g., Roelants et al. 2007; Wiens 2007; Bonett et al. 2013), where life-history shifts likely occurred in the Mesozoic and ecological conditions may have changed considerably since then. A few other salamander clades also show recent transitions to paedomorphosis (e.g., salamandrids, hynobiids, and dicamptodontids; Duellman and Trueb 1986), but in these families the paedomorphic lineages are either too few or too intraspecifically variable to allow for rigorous statistical phylogenetic analyses among species. Only ambystomatids offer similar analytical advantages to spelerpines

(i.e., many seemingly recent origins of paedomorphosis among closely related species; Shaffer and Voss 1996; Weisrock et al. 2006).

In this study, we reconstruct the first nearly comprehensive time-calibrated phylogeny of spelerpines and use this to study the evolution of paedomorphosis and its consequences. We address the following questions. How many times has paedomorphosis evolved in spelerpines? Is the evolution of paedomorphosis reversible? Is the evolution of paedomorphosis associated with invasion of cave habitats, arid surface condition, limited geographic range sizes, and reduced dispersal between biogeographic regions?

Materials and Methods

SAMPLING, DNA SEQUENCING, AND ALIGNMENT

Sampling for DNA analyses included almost all current nominate species of spelerpines (genera: *Eurycea*, *Gyrinophilus*, *Pseudotriton*, *Stereochilus*, and *Urselaperpes*), except the extremely rare *Eurycea robusta* (only known from four specimens; Petranks 1998). Nearly all recognized “subspecies” were also included, as well as previously identified divergent lineages within some species (see Table S1 for vouchers, localities, and GenBank numbers). For species that have paedomorphic and metamorphic populations, we included representatives of both life histories. Specimens were handled in accordance with institutional IACUC protocols at the University of Tulsa and the University of Texas Arlington. DNA was isolated from fresh, frozen, and ethanol preserved tissues using Qiagen DNEasy extraction kits. Polymerase chain reactions (PCRs) were used to amplify portions of two mitochondrial genes, cytochrome *b* (*Cytb*), and NADH dehydrogenase 4 (*Nd4*), and one nuclear gene, recombination activating gene 1 (*Rag1*). Primers and PCR conditions are listed in Table S2. PCR products were checked on 1% agarose gels and treated with ExoSAP-IT (USB Corp., Santa Clara, CA). Cycle sequencing was performed with Big Dye version 3.1 (Applied Biosystems Inc., Foster City, CA). Unincorporated dye terminators were removed from sequencing reactions with Sephadex G-50 (Sigma, St. Louis, MO), or ethanol precipitation and sequenced on ABI 3130xl capillary sequencers. Although data for other genes are available for a limited subsets of spelerpine species (*BDNF*, *POMC*: Vieites et al. 2007; *ND2*: Kozak et al. 2009), we used these three genes to facilitate use of species-tree methods that implicitly require sampling of all species for all sampled genes. Importantly, our phylogenetic estimate for spelerpines is consistent with a previously published phylogeny that included more genes but fewer taxa (Kozak et al. 2009).

Sequences were aligned and edited using Sequencher version 4.8 (Gene Codes, Ann Arbor, MI). Our alignments for each

gene contained 75 terminal taxa (67 spelerpines and eight outgroups). A minority of spelerpine sequences and all outgroup sequences were taken from our previously published data on GenBank. Outgroups (Table S1) included representatives of the three other subfamilies of plethodontids (Bolitoglossinae: *Bolitoglossa*; Hemidactylinae: *Hemidactylium*; Plethodontinae: *Aneides*, *Desmognathus*, *Ensatina*, and *Plethodon*) and an amphiumid (*Amphiuma means*), given that Amphiumidae is consistently placed as sister to Plethodontidae in recent studies (Chippindale et al. 2004; Wiens et al. 2005; Roelants et al. 2007; Zhang and Wake 2009; Pyron and Wiens 2011).

The three genes used were protein coding and their alignments were unambiguous with no missing data, except for a single codon deletion in the *Nd4* sequence of one outgroup species (*Aneides aeneus*). The alignments for each gene were trimmed to have the same length across all taxa (*Cytb*, *Nd4*, and *Rag1* were 674, 633, and 1090 base pairs, respectively).

ESTIMATION OF PHYLOGENY AND DIVERGENCE TIMES

The phylogeny and divergence times were estimated primarily from species-tree analyses, but we first conducted separate analyses of the mitochondrial and nuclear genes. MrModeltest version 2.2 (Nylander 2004) was used to determine the most appropriate model of nucleotide substitution for each codon position for all three genes (Table S3), evaluating the relative fit of each model based on comparisons of likelihood with the AIC. Bayesian analyses implemented in MrBayes version 3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) were run separately for the combined mitochondrial genes (*Cytb* and *Nd4*) and for *Rag1*. We partitioned each gene by codon position (all substitution rate parameters unlinked; following previous partitioning analyses of these genes in plethodontids by Wiens et al. 2006) and applied the models selected above. For each locus (mtDNA and *Rag1*), we ran two replicate searches each with four chains (three hot and one cold) for 20 million generations sampling every 1000 generations. Stationarity was assessed by viewing likelihood values across generations in Tracer version 1.5 (Rambaut and Drummond 2007). The first five million generations (5000 trees) were conservatively discarded as burn-in, which was well beyond stationarity in each case. For each locus, two independent runs converged on the same majority-rule consensus topology, with very similar posterior probabilities. The trees from each locus were also highly concordant with each other. To test for potential problems related to branch length inflation (Brown et al. 2010), we ran both mtDNA and *Rag1* data sets multiple times using a range of branch length priors (exponential with means of 0.01, 0.1, or, 1) and found that this had no effect on the topologies or posterior probabilities.

The species tree and divergence times, based on two unlinked gene trees and clock models (mtDNA and *Rag1*), were estimated using the *BEAST function (Heled and Drummond 2010) in BEAST version 1.6.2 (Drummond and Rambaut 2007). We applied the same best-fitting models (determined earlier) to each partition. Because some nominate species are not monophyletic, we treated each terminal taxon as an individual discrete trait (i.e., 75 “species”). The analyses of each gene tree were based on uncorrelated lognormal molecular clocks and Yule speciation priors across the tree. There are no documented pre-Pleistocene spelerpine fossils identified at the generic or species levels (Holman 2006; Boardman and Schubert 2011). Therefore, we used the crown group age of extant plethodontids as a calibration point. The estimates of this node range from 41 to 99 million years ago, with average estimates at approximately ~73 million years ago (Mueller 2006; Roelants et al. 2007; Vieites et al. 2007; Wiens 2007; Zhang and Wake 2009). We applied a normally distributed calibration prior for the crown group of plethodontids, with a mean of 73 million years ago and standard deviation of 6 million years. This combination of parameters provides a 95% prior distribution between 85 and 65 million years ago, representing a reasonable range of potential dates for the age of this clade based on previous studies. *BEAST analyses were also run twice independently for 20 million generations. Stationarity of likelihood values was evaluated in Tracer and the first 25% of generations were conservatively discarded as burn-in, which was always well beyond stationarity.

EVOLUTION OF PAEDOMORPHOSIS

Taxa were coded as metamorphic or paedomorphic (Table S4) based on literature sources (Petranka 1998; Chippindale et al. 2000; Niemiller et al. 2008; Emel and Bonett 2011). We define a taxon as paedomorphic if the species or population retains aquatic larval traits (e.g., gills) into adulthood. We note that there is general consensus among researchers regarding the life-history mode of each taxon (Petranka 1998; AmphibiaWeb 2013). The evolution of life-history modes (paedomorphic or metamorphic) across the tree (for all nodes) was reconstructed using a Bayesian approach, implemented in the program BayesTraits version 1.0 (www.evolution.rdg.ac.uk) using “BayesMultiState” (Pagel et al. 2004) and a reversible-jump Markov Chain Monte Carlo (rjMCMC) model. To account for uncertainty in the phylogeny, reconstructions were based on all 15,000 post-burn-in Bayesian chronograms from the *BEAST analysis. Life history and ecological data were only included for spelerpines (see Results), and the overall reconstructions were not affected when outgroup data were included (not shown). An exponential reversible-jump hyperprior (rjhp exp) was set from 0.0 to 15, and the rate deviation (ratedev) value was set to 8 (based on several exploratory runs and acceptance rates were between 20% and 40%). Each consensus

reconstruction consisted of five independent runs of 55 million generations (reconstructions), with the first 5 million generations of each run discarded as burn-in (total 250 million post-burn-in generations).

Given the potential for a reversal from paedomorphosis to metamorphosis in *E. troglodytes*, we estimated the probability of paedomorphosis at specific nodes in the phylogeny (a “node-based approach”), and also tested rates of trait gain and loss across the tree (“tree-based approach”; reviewed in Syme and Oakley 2012). For the node-based approach, we used BayesTraits to test for significant reversals in life history by “fossilizing” (fixing) key ancestral nodes to alternative states. We calculated Bayes factors (BF) to compare the differences in the harmonic means from constrained and unconstrained analyses. Given the most prominent potential reversal from paedomorphosis to metamorphosis is among Edwards Plateau *Eurycea*, we fossilized two deep nodes in this clade to be metamorphic: (1) the ancestor of all Edwards Plateau *Eurycea* (Fig. 3 node b; *Paedomolge* clade under an unranked Phylocode taxonomy, Hillis et al. 2001); and (2) the ancestor of the clade of Edwards Plateau *Eurycea* occurring south of the Colorado River (Fig. 3 node c; *Notiomolge* clade; Hillis et al. 2001). Bayes factors >2 were considered positive evidence and >5 considered strong evidence against these alternative hypotheses (Pagel and Meade 2006).

For the tree-based approach, we first calculated the rate of change (gain and loss) of life-history mode (metamorphosis: M; paedomorphosis: P) on the chronogram of spelerpines using the Mk-2 model in Mesquite version 2.73 (Maddison and Maddison 2008). We used the consensus *BEAST chronogram, and we set the states at the base of the tree to equal probabilities (Goldberg and Igic 2008; Wiens 2011). We compared the fit of the unconstrained model to one in which the rate of trait re-evolution (paedomorphosis to metamorphosis) was set to zero. We also performed these tests considering the effects of speciation and extinction on our character reconstructions using a binary state speciation and extinction model (BiSSE; Maddison et al. 2007) in Mesquite. Tests of reversals were otherwise the same as described earlier except the rate of the constrained model was set to an extremely small number ($1.0e^{-17}$; Mesquite does not accept zero for this analysis). These reversal tests were also performed for ecology (surface-dwelling: S; cave-dwelling: C). ΔAIC values ≥ 4 between models were considered moderately strong support for rejecting irreversibility, values ≥ 10 were considered very strong support, and values <3 were considered to offer negligible support (Burnham and Anderson 2002; Goldberg and Igic 2008). We also used BiSSE to perform exploratory estimates of speciation and extinction rates for each life history and ecological state, but our number of taxa (67) is too small to effectively test if rate differences are significant (Davis et al. 2013).

CAVE-DWELLING AND PAEDOMORPHOSIS

We performed similar analyses for the evolution of cave-dwelling, which may be associated with paedomorphosis. Taxa were coded as surface dwelling or cave dwelling (Table S4) based on literature sources (Petranka 1998; Chippindale et al. 2000; Niemiller et al. 2008). We acknowledge that there is a continuum between species that do not occur in caves, species with some populations that occur in caves and others that do not (e.g., *Eurycea lucifuga*), species that always occur in caves but only for part of their lives (e.g., adult *E. spelaea*), and those that occur only in caves (e.g., *Eurycea rathbuni*; Petranka 1998). We coded species as cave-dwelling only if they appeared to be obligate cave-dwellers for at least part of their life cycle, and especially if they show morphological traits associated with living in caves (reduced eyes and pigmentation; Petranka 1998; Wiens et al. 2003; AmphibiaWeb 2013). For the Edwards Plateau *Eurycea*, we treated *E. rathbuni*, *Eurycea tridentifera*, and *Eurycea waterlooensis* as cave-dwelling; although invasion of caves has occurred in other members of the group and such populations show varying degrees of troglomorphy (Bendik et al. 2013). These three species (plus *E. robusta*, a member of the *Typhlomolge* clade that could not be included due to habitat inaccessibility) appear to be the only obligate cave-dwellers (Chippindale et al. 2000). Other cave-dwelling spelerpines include *Eurycea spelea*, *Eurycea wallacei*, *Gyrinophilus gulolineatus*, *Gyrinophilus palleucus*, and *Gyrinophilus subterraneus*.

To determine if life history is associated with cave-dwelling, we conducted maximum likelihood and Bayesian tests of correlated (discrete) character evolution in BayesTraits (Pagel and Meade 2006). These methods test the null hypothesis of independent evolution of two discrete characters, by comparing a model where the rate of change of one character is independent of changes in the other character, versus a model where state changes of each character are dependent (Pagel 1994). For Bayesian analyses, we used the rjMCMC parameters and run lengths described earlier (using 15,000 trees), and Bayes factors were used to compare the alternative models. We also used BayesTraits to examine dependent rate parameters and test the temporal order of changes (i.e., did life-history transitions precede shifts in ecology, or vice versa; Pagel 1994; Valenzuela and Adams 2011). This was performed by comparing the likelihood of a dependent model with unconstrained transition rates (eight parameters) to a dependent model where transition rates from surface- to cave-dwelling were equal to those of metamorphosis to paedomorphosis (seven parameters; $q_{12} = q_{13}$; Pagel 1994). We also tested the alternative form of this model in which transition rates from cave- to surface-dwelling were constrained to equal those of paedomorphosis to metamorphosis (seven parameters; $q_{42} = q_{43}$; Pagel 1994). These models were analyzed in both maximum likelihood and Bayesian contexts and compared with likelihood ratio tests and ΔAIC .

CLIMATE AND PAEDOMORPHOSIS

We tested the hypothesis that paedomorphosis is more likely to evolve in regions with conditions that are relatively inhospitable for terrestrial adults (e.g., arid climatic conditions). This hypothesis is suggested for spelERPINES by the fact that paedomorphic surface-dwelling lineages occur primarily at the drier edges of the range of the subfamily (i.e., the Edwards Plateau and the Interior Highlands; Petranks 1998). To test this hypothesis, we started with climatic data for spelERPINE species from Kozak and Wiens (2010). These data are based largely on georeferenced localities from the MVZ and USNM museum collections, supplemented with additional localities (details provided in Methods S1). Climatic data are from the WorldClim database (Hijmans et al. 2005), at a spatial scale of $\sim 1 \text{ km}^2$. However, data were lacking or limited for some species and populations. Therefore, we used the WorldClim database at the same spatial scale and obtained new climatic data for these species, mostly using localities from published studies (Methods S1).

To examine the relationship between climate and paedomorphosis in a phylogenetic context, we used the phylogenetic logistic regression approach of Ives and Garland (2010). This method uses a two-state Markov process to model the evolution of the binary-dependent variable (here, metamorphic taxa were coded as “0” and paedomorphic taxa as “1”), and assumes that values for the continuous independent variables are known properties of extant species. We used two measures of climate as independent variables: Bio12 (annual precipitation) and Bio17 (precipitation of the driest quarter), each averaged over all localities for each taxon. We focused on Bio12 as a standard measure of precipitation, and we used Bio17 to reflect rainfall during the driest portion of the year (low rainfall during this period may be most problematic for the persistence of terrestrial adults). Climatic data for each lineage are summarized in Appendix S1. We analyzed each predictor variable separately, and standardized each to have a mean of 0 and a standard deviation of 1 prior to analysis. Following the recommendations of Ives and Garland (2010), we used 2000 bootstrap replicates to obtain our estimates for the slope parameters of the regressions (β_1) and 95% confidence intervals for these estimates. A confounding factor in our analyses is that climatic conditions on the surface are potentially irrelevant for cave-dwelling species, many of which are paedomorphic. Therefore, we repeated these analyses after removing the seven cave-dwelling taxa (*Eurycea rathbuni*, *E. spelea*, *E. tridentifera*, *E. wallacei*, *E. waterloensis*, *Gyrinophilus gulolineatus*, *G. pallescens*, *G. subterraneus*).

PAEDOMORPHOSIS, DISPERSAL, AND RANGE SIZE

The distributions of ancestral spelERPINE lineages were reconstructed using likelihood-based inference in LAGRANGE (Ree et al. 2005; Ree and Smith 2008), to determine the number of

dispersal events between regions and whether this is influenced by paedomorphosis. The analysis takes into account topology and branch lengths, and was performed on the consensus species-tree chronogram. The geographic regions used were (1) Appalachian Mountains (and adjacent regions), (2) Coastal Plain (low elevation regions adjacent to the Atlantic Ocean and Gulf of Mexico in southeastern North America), (3) Interior Highlands (Ouachita Mountains and the Ozark Plateau), and (4) Edwards Plateau of Central Texas (Fig. 1). These represent the major areas of species endemism for spelERPINE salamanders (Petranks 1998). The analysis allowed ancestors to occur in any two areas and there were no constraints on dispersal between regions.

Given the estimated dispersal events across the tree, we then tested whether dispersal events were more common for paedomorphic or metamorphic ancestors (i.e., does one life history mode show a higher propensity for dispersal?). A one-tailed binomial statistic was used to test if significantly more dispersals were by metamorphic than paedomorphic ancestors. Based on our ancestral state reconstructions, we set the expected probability to be either the proportion of ancestral nodes in each state (65:35, metamorphic:paedomorphic), or the proportions of branch lengths in each state (82:18, metamorphic:paedomorphic with transitional branch lengths evenly divided between states). Our observations were the reconstructed life histories of dispersing hypothetical ancestors. We counted only ancestors whose life history was consistently reconstructed with high support (proportional probability or proportional likelihood > 0.75) using both Bayesian and likelihood methods. This test would not be effective for surface versus cave-dwellers given that 90% of ancestral nodes and 94% of ancestral branch lengths are estimated to have been surface-dwellers, so it would be expected that most or all dispersals would have been by surface-dwellers (and they were).

We used phylogenetic analyses of variance (Garland et al. 1993) in the R package *phytools* (Revell 2012) to test for significant differences in the geographic range sizes (dependent variable) of species with different life histories (metamorphic vs. paedomorphic) and different habitats (surface vs. cave), based on 10,000 simulations. The geographic range areas (km^2) of 39 spelERPINE taxa were determined using ArcMap 9 (Table S4), based on county-scale shape files from the IUCN Red List (2011). Area was \log_{10} -transformed for analyses. Many named plethodontid species show strong geographic genetic substructure, and likely include many undescribed species (Kozak et al. 2006; Timpe et al. 2009; Emel and Bonett 2011). Given that species boundaries have not been delineated for all nominal spelERPINE species, for wide-ranging taxa we measured only the geographic range sizes of genetic subgroups (e.g., *Eurycea bislineata* complex; Kozak et al. 2006). We did not include four wide-ranging species that have not been the subject of detailed phylogeographic studies (*Gyrinophilus porphyriticus*, *Pseudotriton montanus*,

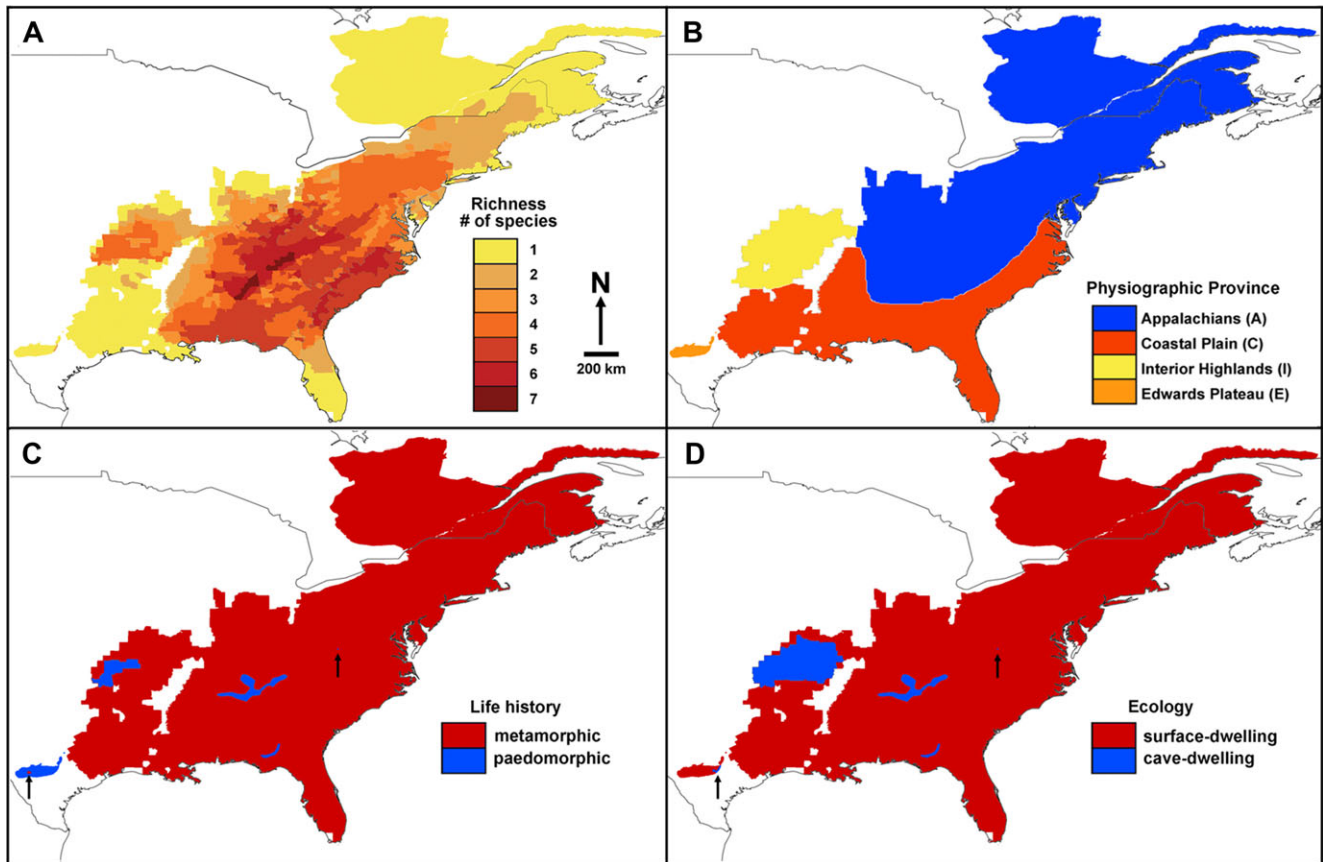


Figure 1. Diversity and distribution of spelerpine plethodontid salamanders in eastern North America. (A) Species richness of spelerpines, (B) physiographic regions used in biogeographic analyses, (C) paedomorphic taxa (blue, which are codistributed with metamorphic taxa except in the Edwards Plateau), and (D) cave-dwelling taxa (blue, which are always codistributed with surface-dwelling taxa). Distributions are based on IUCN Red List shape files assembled in ArcMap 9.0.

Pseudotriton ruber, and *Stereochilus marginatus*). These are all metamorphic, surface-dwelling taxa with large distributions and their inclusion would only increase the significance of our results.

Results

PHYLOGENY

Bayesian phylogenetic analyses based on mitochondrial DNA (*Nd4* and *Cytb*; Fig. S1) and nuclear DNA (*Rag1*; Fig. S2), and the species tree (Fig. 2) are largely congruent and show strong support for major nodes. As in previous phylogenetic analyses (Chippindale et al. 2004; Mueller et al. 2004; Kozak et al. 2009), we find strong support for monophyly of Spelerpinae (*Eurycea*, *Gyrinophilus*, *Pseudotriton*, *Stereochilus*, and *Ursperlperes*) within Plethodontidae. All analyses also support monophyly of *Eurycea* and *Gyrinophilus* (*Stereochilus* and *Ursperlperes* are monotypic, and monophyly of *Pseudotriton* was not supported in most analyses). *Ursperlperes* is always recovered as the sister taxon of *Eurycea* (see also Camp et al. 2009), but this is only well supported in the *Rag1* and species tree analyses. *Eurycea* com-

prises several well-supported clades. The *Eurycea multiplicata* complex, endemic to the Interior Highlands (Bonett and Chippindale 2004), is monophyletic and sister to all other *Eurycea*. The *E. bislineata* complex (Kozak et al. 2006) and *Eurycea longicauda* complex are each monophyletic. The Georgia blind salamander (*E. wallacei*, formerly *Haideotriton*) is placed either within the *E. bislineata* complex (mtDNA and species tree) or sister to the *E. longicauda* complex (*Rag1*). The *Eurycea quadridigitata* group contains multiple divergent lineages across the Coastal Plain. In the species tree (Fig. 2), the westernmost populations are most closely related to *Eurycea* from the Edwards Plateau of Texas (i.e., the Edwards Plateau *Eurycea* appear phylogenetically nested within *E. quadridigitata*). This is consistent with recent analyses of the *E. quadridigitata* group by Lamb and Beamer (2012). However, our *Rag1*-only analysis places the eastern *E. quadridigitata* group sister to the *E. bislineata* complex (posterior probability = 0.75), and mtDNA data alone place western *E. quadridigitata* populations inside the clade of Edwards Plateau *Eurycea* (but with only weak support; posterior probability = 0.56).

Similar to recent phylogenetic analyses (Chippindale et al. 2004; Mueller et al. 2004; Kozak et al. 2009), we find

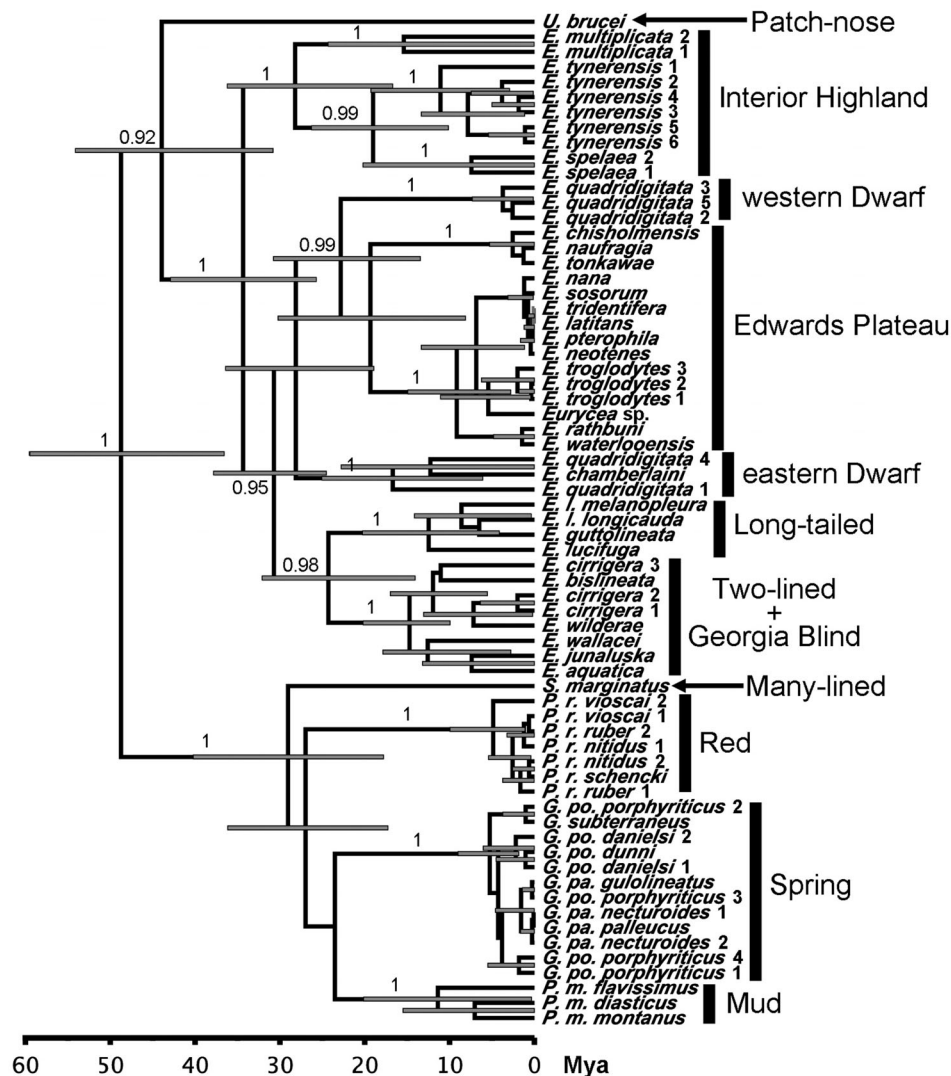


Figure 2. Bayesian species tree chronogram for spelerpine salamanders. The species tree and divergence times estimates from *BEAST are based on mitochondrial DNA (*Cytb* and *Nd4*) and a nuclear gene (*Rag1*). The number subtending each node is the posterior probability for the clade (≥ 0.90 shown), and bars indicate 95% highest posterior density intervals on divergence dates. For clarity, support for nodes within major clades is not shown. *E.* = *Eurycea*; *G.* = *Gyrinophilus*; *P.* = *Pseudotriton*; *S.* = *Stereochilus*; *U.* = *Urselaperpes*.

that *Gyrinophilus*, *Pseudotriton*, and *Stereochilus* form a clade, but the relationships among these lineages are not clear. The two nominate species of *Pseudotriton* (*Pseudotriton ruber* and *Pseudotriton montanus*) are highly divergent from one another. *Pseudotriton* is monophyletic (but with weak support; posterior probability = 0.71) in the mtDNA tree, and is not monophyletic in the *Rag1* or species trees (but monophyly of *Pseudotriton* is supported in concatenated analyses of multiple nuclear and mitochondrial genes; Kozak et al. 2009).

EVOLUTION OF PAEDOMORPHOSIS

Bayesian reconstructions show that ancestral spelerpines were likely metamorphic and that there were several independent shifts to paedomorphosis throughout the history of this group (Fig. 3).

These shifts include: (a) an ancient shift in the ancestor of Edwards Plateau *Eurycea*; (b) shifts in multiple populations of *E. tynerensis* in the western Ozarks (see also Bonett and Chippindale 2004); (c) single shifts in the ancestors of *E. wallacei* on the Coastal Plain and *G. subterraneus* in the Appalachians; and (d) potentially multiple instances among populations of *G. gulolineatus* and *G. palleucus* on the Cumberland Plateau of the Appalachians (see also Niemiller et al. 2008).

Using node-based approaches we find strong support for the re-evolution of metamorphosis in *E. troglodytes* after several million years of paedomorphosis. Bayesian reconstructions favor a paedomorphic ancestor as old as ~22 million years ago (proportional probability = 0.71; the ancestor of Edwards Plateau *Eurycea* and western *E. quadridigitata*; Fig. 3 node a).

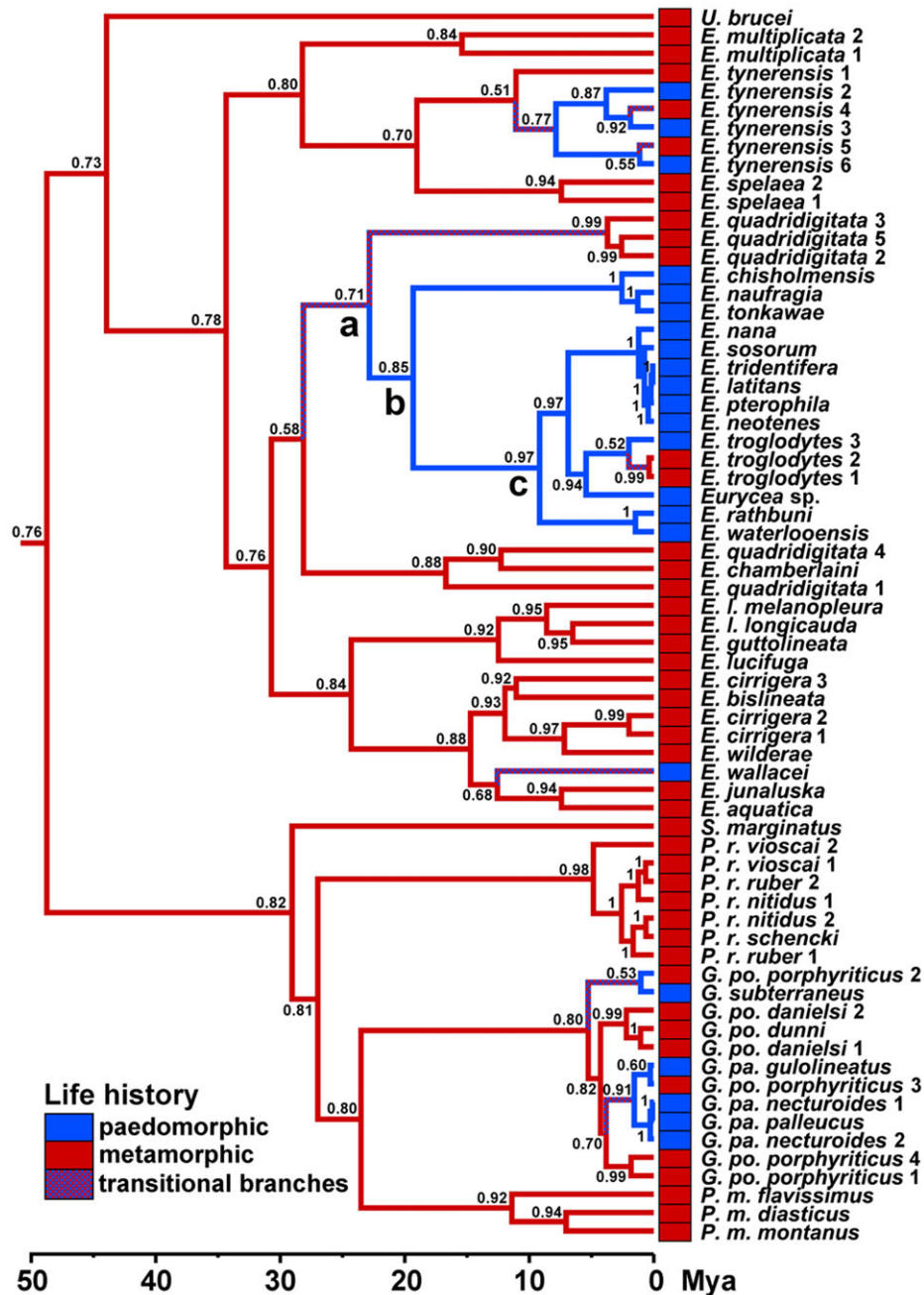


Figure 3. Ancestral life-history reconstruction of spelerpines. Bayesian reconstruction of life history (metamorphosis and paedomorphosis) using BayesMultistate in BayesTraits with a reversible jump MCMC model. The number subtending each node is the posterior probability for the more likely state (indicated with colors): metamorphic (red) and paedomorphic (blue). Hatched branches represent inferred transitions between states. The chronogram is based on Bayesian species tree analysis in *BEAST (Fig. 2). Lowercase letters denote three clades: (a) Edwards Plateau *Eurycea* plus western *E. quadridigitata*; (b) *Paedomolge*, all Edwards Plateau *Eurycea*; and (c) *Notiomolge*, Edwards Plateau *Eurycea* from south of the Colorado River. *E.* = *Eurycea*; *G.* = *Gyrinophilus*; *P.* = *Pseudotriton*; *S.* = *Stereochilus*; *U.* = *Urselaperpes*.

However, fossilizing ancestral nodes to metamorphosis only has a significant effect on the reconstruction when it is applied to the ancestor of the *Notiomolge* clade of Edwards Plateau *Eurycea* (node c, Fig. 3; ~9 million years ago; BF = 6.45). Likelihood-based reconstructions also suggest that this ancestor (node c) was

paedomorphic (proportional probability = 0.98; Fig. S3). Unconstrained rate models that allow for reversals to metamorphosis are a significantly better fit to the data than rate models with the probability of reversal set to zero ($\Delta\text{AIC} = 26.229$; Table 1). There is also strong support for reversal when effects of life history on

Table 1. Results of tree-based tests of trait reversals across the phylogeny of spelerpines for life history (M = metamorphic; P = paedomorphic) and ecology (S = surface; C = cave-dwelling). In each case, unconstrained two rate models (Mk2; left column) are compared to constrained analyses in which reversals (P to M or C to S) are not allowed (0) or set to an extremely small number ($1.00E-17$; right column). q_{MP} and q_{SC} are forward rates and q_{PM} and q_{CS} are rates of reversal. ΔAIC is used to compare the differences in fit for models with and without reversals. BiSSE analyses also incorporate parameters for speciation/extinction (a) and net diversification (r) for each character.

Life history (metamorphic and paedomorphic)		
	Mk2 unconstrained	Mk2 constrained
–ln L	33.7954	47.9101
AIC	71.5908	97.8203
q_{MP}	0.0277 (estimated)	0.0186 (estimated)
q_{PM}	0.1072 (estimated)	0 (set)
$\Delta AIC = 26.229$		
	BiSSE unconstrained	BiSSE constrained
–ln L	244.4514	251.9813
AIC	492.9028	505.9626
q_{MP}	0.0466 (estimated)	0.0586 (estimated)
q_{PM}	0.0591 (estimated)	$1.00E-17$ (set)
a_M	$1.60E-5$	$7.16E-5$
a_P	0.9570	0.9994
r_M	0.0866	0.1103
r_P	0.0180	$3.63E-4$
$\Delta AIC = 13.059$		
Ecology (surface and cave dwelling)		
	Mk2 unconstrained	Mk2 constrained
–ln L	29.0609	35.9243
AIC	62.1219	73.8487
q_{SC}	0.0711 (estimated)	0.0108 (estimated)
q_{CS}	0.4409 (estimated)	0 (set)
$\Delta AIC = 11.727$		
	BiSSE unconstrained	BiSSE constrained
–ln L	243.2947	248.1486
AIC	490.5894	498.2972
q_{SC}	0.4605 (estimated)	0.0355 (estimated)
q_{CS}	2.8781 (estimated)	$1.00E-17$ (set)
a_S	0.3069	0.1878
a_C	0.9984	0.9998
r_S	0.0702	0.0993
r_C	$7.13E-4$	$7.50E-5$
$\Delta AIC = 7.708$		

speciation and extinction are accounted for using the BiSSE model ($\Delta AIC = 13.059$; Table 1).

ORIGIN OF CAVE-DWELLING LINEAGES AND ASSOCIATIONS WITH PAEDOMORPHOSIS

Bayesian reconstructions (Figs. 4, S4) show that the ancestor of spelerpines was surface-dwelling, and that there have

been multiple independent invasions of cave habitats in *Eurycea* and *Gyrinophilus*. These shifts to cave-dwelling include: (a) at least two shifts on the Edwards Plateau, the *Typhlomolge* clade (*E. rathbuni* and *E. waterlooensis*) and also *E. tridentifera* (Chippindale et al. 2000; Wiens et al. 2003); (b) single shifts in *E. wallacei* (formerly *Haideotriton*) on the Gulf Coastal Plain, *E. spelaea* on the Ozark Plateau (Bonett and Chippindale 2004), and *G. subterraneus* in the Appalachians; and (c) potentially multiple instances of cave-dwelling among populations of *G. gulosineatus* and *G. pallescens* on the Cumberland Plateau of the Appalachians (see also Niemiller et al. 2008).

There are no clear reversals from cave-dwelling to surface-dwelling habitat use, based solely on ancestral state reconstruction (a node-based approach). However, unconstrained rate models that allow for reversals are a significantly better fit to the data than models where the probability of reversal is set to zero ($\Delta AIC = 11.726$; Table 1). This may largely be driven by recent variation in the *G. pallescens* and *G. porphyriticus* clade, which renders some ancestral states equivocal. Recent ancestors of this group may have reversed to surface-dwelling after brief periods of troglotic life. Models allowing reversals when analyzed with the BiSSE model are also moderately supported ($\Delta AIC = 7.708$; Table 1).

Paedomorphosis is highly correlated with cave-dwelling. Our Bayesian test of correlated evolution showed that 99.998% of 250 million generations (from five separate runs of 50 million post-burn-in generations each sampled across 15,000 trees) support the dependent model. There is also significant evidence of a correlation when comparing the harmonic means of independent and dependent analyses ($BF = 10.48$). The only taxa that do not follow this association are some surface-dwelling paedomorphs from the Edwards Plateau, surface-dwelling paedomorphic *E. tynerensis* from the Ozark Plateau, and cave-dwelling *E. spelaea* that metamorphose. *Eurycea lucifuga* is a metamorphic species that often occurs in caves as adults, so we initially did not code it as cave-dwelling. However, the association between cave-dwelling and paedomorphosis remains significant even when *E. lucifuga* is coded as cave-dwelling ($BF = 8.76$).

Under the unconstrained dependent model, the rate of change from metamorphosis to paedomorphosis ($q_{13} = 0.0298$) is more than 14 times higher than the rate of change from surface-dwelling to cave-dwelling ($q_{12} = 0.0021$). However, tests of the temporal order of changes show that there is no significant difference between the unconstrained model and the model with the rates of these transitions constrained to be equal ($L_{\text{unconstr.}} = -52.5237$; $L_{q_{12}=q_{13}} = -53.3615$; $X^2_{df=1} = 1.6754$; $P < 0.1955$; $\Delta AIC = 0.3243$ in favor of the constrained model). This same pattern is also observed if we examine the rates of loss of the ancestral states (i.e., the rate of change from paedomorphosis to metamorphosis and cave-dwelling to surface-dwelling are

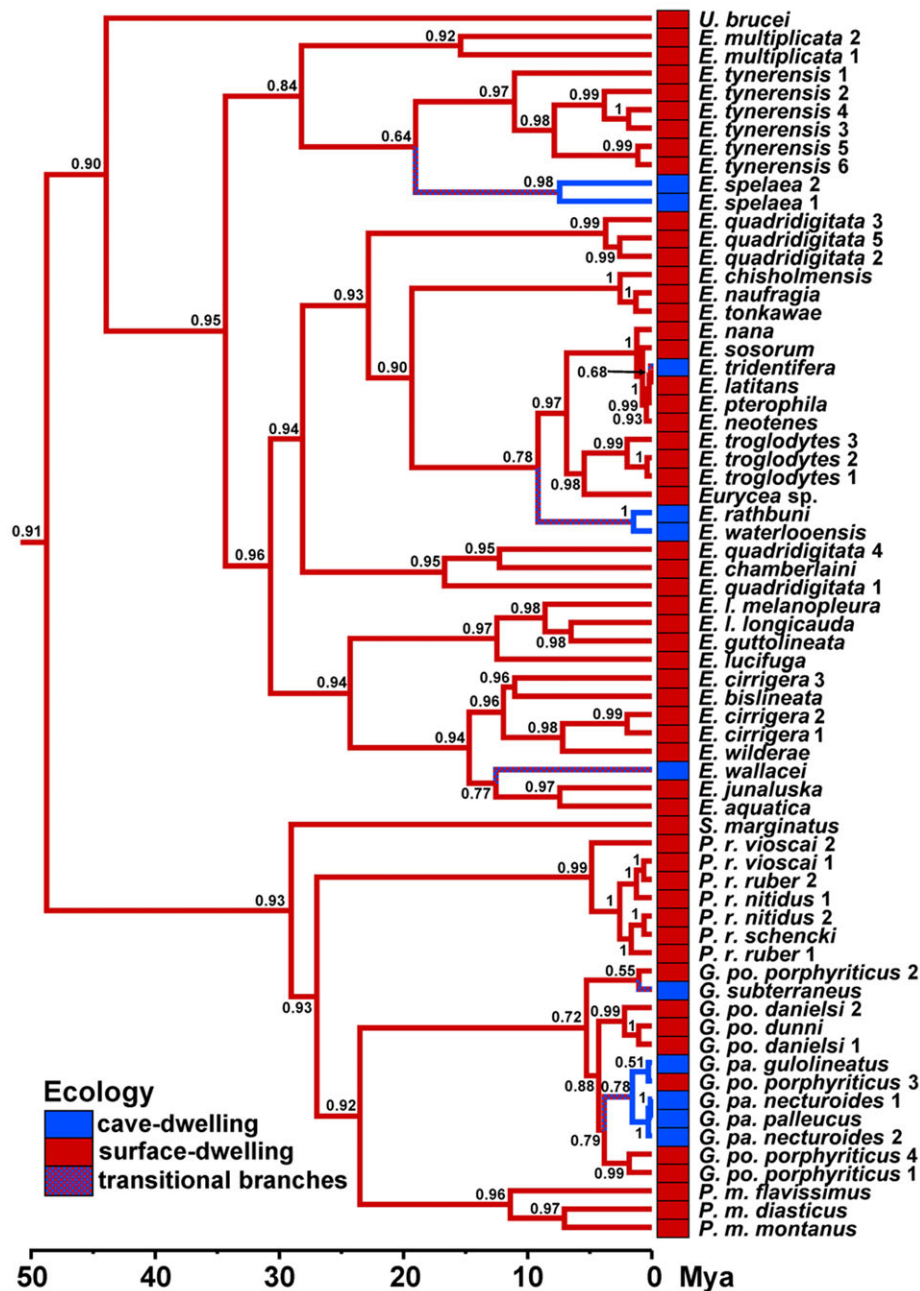


Figure 4. Ancestral reconstruction of habitat. Bayesian reconstruction of habitat (surface-dwelling and cave-dwelling) using BayesMultistate in BayesTraits and a reversible jump MCMC model. The number subtending each node is the posterior probability for the more likely state (indicated with colors): surface-dwelling (red) and cave-dwelling (blue). Hatched branches represent nodes on which there is an inferred transition between states. The chronogram is based on Bayesian species tree analysis in *BEAST (Fig. 2). *E.* = *Eurycea*; *G.* = *Gyrinophilus*; *P.* = *Pseudotriton*; *S.* = *Stereochilus*; *U.* = *Urspeleperpes*.

constrained to be equal; $L_{q42=q43} = -52.9439$; $X^2_{df=1} = 0.8404$; $P < 0.3593$; $\Delta AIC = 1.1596$ in favor of the constrained model). Comparing these models using Bayesian estimation yielded similar patterns. Therefore, our results show that although there is a strong correlation between life history and ecology, we cannot determine which trait likely changed first, and we could not reject (or support) simultaneous evolution of these traits.

CLIMATE AND PAEDOMORPHOSIS

The results of our phylogenetic logistic regressions indicate that both annual precipitation and precipitation of the driest quarter significantly predict developmental mode, with more arid environments strongly associated with paedomorphosis (Table 2; Fig. 5). These results are consistent whether cave-dwelling taxa are included or not. With cave-dwelling taxa removed, the estimated

Table 2. Results of phylogenetic logistic regression analysis of the relationship between climate and paedomorphosis based on 2000 bootstrap replicates.

Climatic variable	95%		P-value
	Estimated slope	confidence interval	
All taxa (46 taxa)			
Bio12 (annual precipitation)	-1.3686	(-2.5461, -0.58778)	0.001
Bio17 (driest quarter precipitation)	-1.2676	(-2.5648, -0.46114)	<0.001
Surface only (39 taxa)			
Bio12 (annual precipitation)	-2.0897	(-4.0255, -1.0259)	<0.001
Bio17 (driest quarter precipitation)	-2.1281	(-4.2582, -0.99545)	<0.001

slope parameters are higher, but with wider confidence intervals (Table 2).

PAEDOMORPHOSIS, DISPERSAL, AND RANGE SIZE

The raw geographic range areas of metamorphic lineages (mean = 199,000 km² ± 334,000; $n = 22$ taxa) are on average 75 times larger than those of paedomorphic lineages (mean = 2600 km² ± 4900; $n = 17$ taxa), and this difference is highly significant when accounting for phylogeny (phylogenetic ANOVA: $F_{(1,37)} = 51.0104$; $P < 0.0001$). Consistent with this pattern, dispersal between the four regions has been primarily (or exclusively) by metamorphic ancestors (all nine dispersals where ancestral life

history could be determined; $P < 0.02$; Figs. 3, 6, S3). This pattern is still significant even if we do not count some recent dispersals that only slightly cross into an adjacent region (e.g., *Eurycea chamberlaini* and *Eurycea guttolineata*). If we use branch lengths (time) of each life history to estimate the null probability (82:18, metamorphic : paedomorphic), then the difference in dispersal events is not significant ($P < 0.13$). Thus, there is a significant difference in dispersal between life histories per species, but not per unit of time. However, the use of time implicitly assumes that time does not differ between life histories. Yet, branch lengths for metamorphic lineages (average 6.9 million years) are significantly longer than for paedomorphic lineages (average 2.4 million years; t -test $P < 0.0001$). Therefore, the use of time may bias the test, because paedomorphic life histories may both limit dispersal and decrease branch lengths (e.g., due to extinction of older lineages), or limited dispersal may itself increase extinction. Importantly, there are no definitive cases of paedomorphic ancestors dispersing among these regions. If we further subdivide these regions (e.g., subdividing the Interior Highlands into Ozark Plateau and Ouachita Mountains, or Coastal Plain into Atlantic and Gulf), we would only increase the inferred number of cases of dispersal by metamorphic lineages without adding any cases of dispersal by paedomorphic lineages. The speciation rate is approximately five times higher for paedomorphs than metamorphs, but extinction rate for paedomorphs is more than 15,000 times higher (Table S5), which may be a consequence of limited range sizes and dispersal capabilities.

The raw geographic range areas of surface-dwelling lineages (mean = 145,000 km² ± 299,000; $n = 30$ taxa) are on average 20 times larger than those of cave-dwelling lineages (mean = 7200 km² ± 10,000; $n = 9$ taxa), but this difference is not significant (phylogenetic ANOVA: $F_{(1,37)} = 6.114$; $P = 0.1401$).

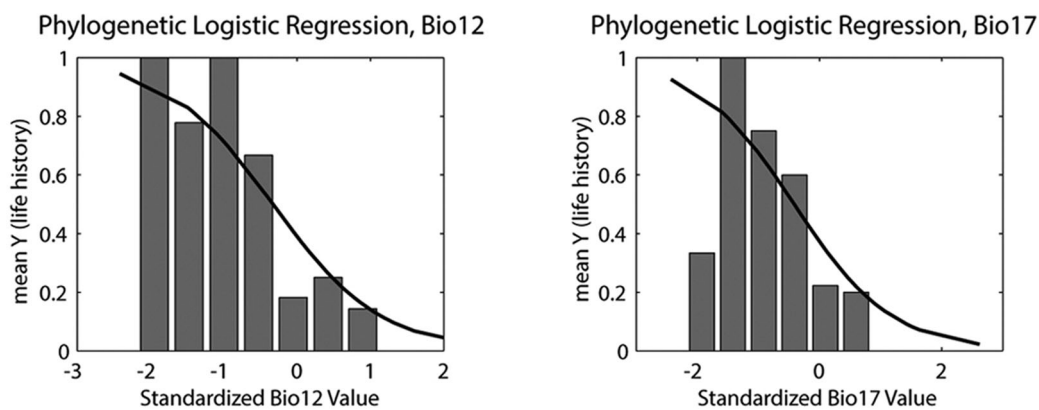


Figure 5. Results of phylogenetic logistic regression between life-history mode (0 = metamorphic, 1 = paedomorphic) and the independent variables Bio12 (annual precipitation) and Bio17 (precipitation of the driest quarter). Independent variables are standardized to have a mean of 0 and a standard deviation of 1. Regression lines are plotted using the coefficients returned by generalized estimating equation approximation for phylogenetic logistic regression with the Firth correction (for further explanation, see Ives and Garland 2010). Figures modified from those provided by the PLogReg script of Ives and Garland (2010) in MatLab 2009a.

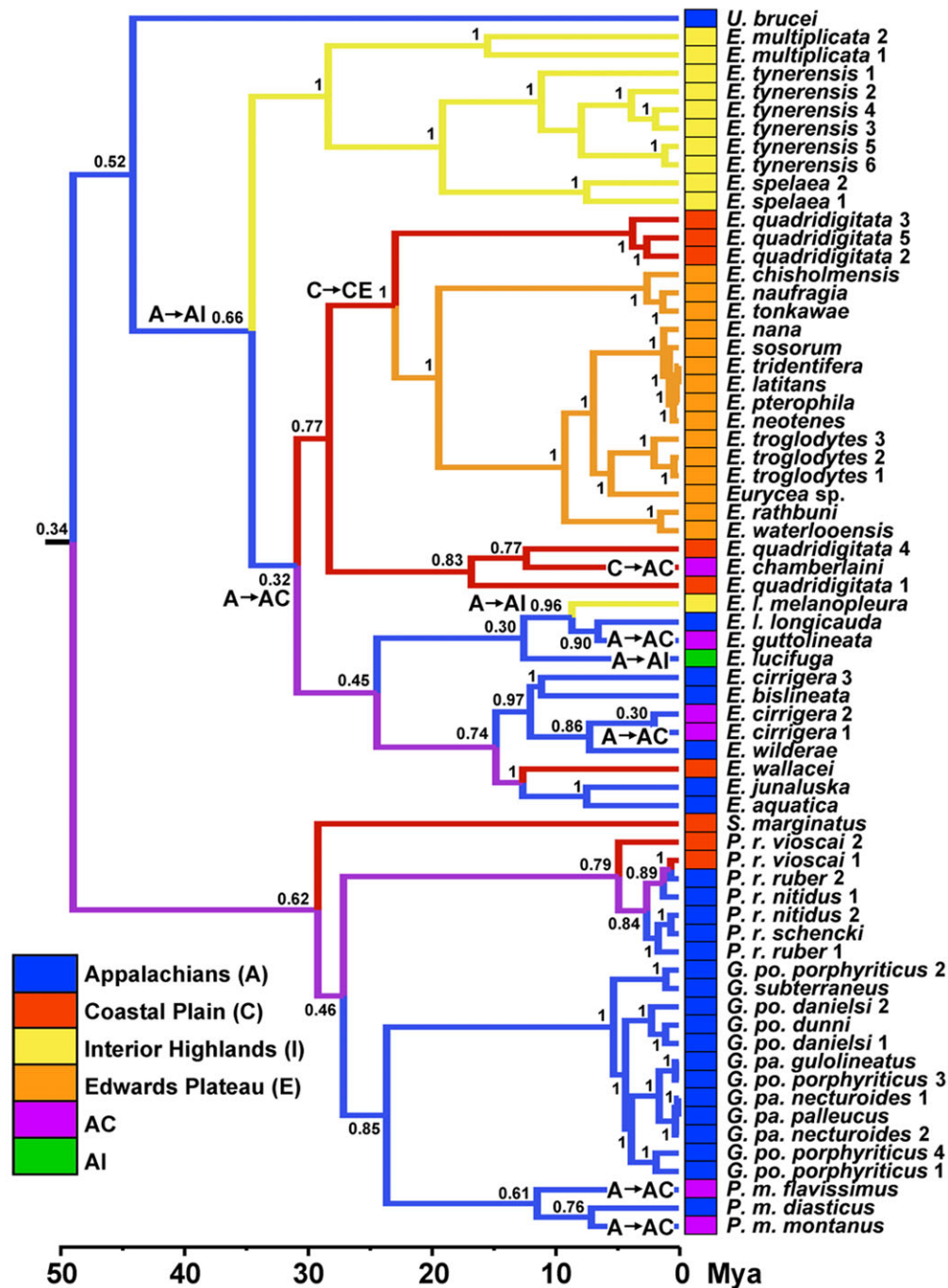


Figure 6. Ancestral area reconstruction for spelerpine salamanders. Likelihood reconstruction in Lagrange considering four discrete regions: Appalachians and adjacent uplands (blue), Coastal Plain (red), Interior Highlands (yellow), and Edwards Plateau (orange). Some extant species and ancestors likely occurred in two regions: Appalachians including adjacent uplands and Coastal Plain (purple), and Appalachians including adjacent uplands and Interior Highlands (green). The number subtending each node indicate the highest split probability for the ancestral area. Simultaneous distributions in other areas were not recovered with high likelihood for any node. The chronogram is based on Bayesian species tree analysis in *BEAST (Fig. 2). *E.* = *Eurycea*; *G.* = *Gyrinophilus*; *P.* = *Pseudotriton*; *S.* = *Stereochilus*; *U.* = *Urselaperpes*.

This may be due to the high variance in range areas within both groups, and because several surface-dwelling paedomorphs have small ranges. All dispersals between regions have been by surface-dwelling lineages, although this is not unexpected given

that 90% of ancestors and 94% of branch lengths are estimated to be surface-dwellers. Similar to life history, surface-dwelling branch lengths (average 5.9 million years) are significantly longer than cave-dwelling branch lengths (average

1.7 million years; t -test $P < 0.01$). There are no definitive cases of cave-dwelling lineages dispersing among these biogeographic regions. The cave salamander (*E. lucifuga*) recently dispersed between the Appalachians and Interior Highlands (Timpe et al., unpubl. data), but is not an obligate cave-dweller. Similar to life history, the speciation rate is approximately five times higher for cave-dwellers than for surface-dwellers, but the extinction rate for cave-dwellers is more than two million times higher than for surface dwellers (Table S5).

Discussion

Paedomorphosis in salamanders has been of considerable interest to evolutionary biologists for centuries (Gould 1977). However, few previous studies have used phylogenies to address the basic evolutionary patterns of gain and loss of paedomorphosis, and none have tested its potential ecological correlates and consequences in a phylogenetic context. Therefore, many basic questions about the evolution of paedomorphosis have remained unanswered. Here, we use a new time-calibrated phylogeny for spelerpines to address these questions. Our results show multiple origins of paedomorphosis in spelerpines. They also show evidence that paedomorphosis can be reversed over relatively long time scales (i.e., several million years), suggesting that metamorphosis and ancestral adult traits can re-evolve after being evolutionarily “lost” (or not expressed). Our results also show two important ecological correlates of the origin of paedomorphosis and two important consequences. Theory suggests that paedomorphosis will evolve when ecological conditions are unfavorable in terrestrial environments, but remain favorable in the larval, aquatic environment (Wilbur and Collins 1973). In accordance with these predictions, we find that paedomorphosis is associated with both dwelling in caves and occurrence in more arid climates. Furthermore, we find that paedomorphosis is associated with reduced dispersal among geographic regions and smaller geographic range sizes. We discuss these findings in more detail below.

LOSS AND RE-EVOLUTION OF METAMORPHOSIS?

A particularly surprising result of our study is that paedomorphosis may be reversible, even after relatively long time scales. Over the last decade, several studies have shown strong evidence for the reversibility of a wide range of morphological and life-history characteristics (e.g., Chippindale et al. 2004; Brandley et al. 2008; Kohlsdorf et al. 2010; Lynch and Wagner 2010; Wiens 2011), contrary to Dollo’s law of irreversibility (Dollo 1893). However, the re-evolution of metamorphosis in spelerpines is especially intriguing for two main reasons. The first is the time scale. Here we show that metamorphic populations of *E. troglodytes* from the western Edwards Plateau are phylogenetically nested in a clade (minimally the *Notiomolge* clade of Edwards Plateau *Eurycea*), which

otherwise contains exclusively paedomorphic taxa (Fig. 3). The *Notiomolge* clade is approximately 10 million years old, and the metamorphic populations of *E. troglodytes* are approximately 2 million years divergent from their most closely related paedomorphic populations. Thus, our results suggest that metamorphosis was absent for minimally 8 million years before re-evolving in *E. troglodytes*. Bayesian reconstructions show a paedomorphic ancestor as old as 22 million years ago (and this is the most intuitive pattern given the phylogeny; Fig. 3), but we acknowledge that the support for paedomorphosis at deeper nodes is more ambiguous (proportional probability = 0.71, and no support in ML reconstructions). The time scale is interesting because genes that are no longer used are thought to be rendered irreversible by mutations after >10 million years (Marshall et al. 1994). There are now many examples of traits re-evolving after longer time scales (reviewed in Wiens 2011), and this may be possible because genes remain intact by serving alternative functions.

The second reason why this finding is so intriguing is the large number of morphological traits that are involved. In salamanders, complete metamorphosis involves the absorption of gills and tailfin, remodeling of the cartilaginous hyobranchial apparatus, many developmental changes to the skeleton and skin, and numerous physiological modifications (Duellman and Trueb 1986; Rose 1996). As in other amphibians, many of these processes are governed or at least initiated by thyroid and stress hormones (Rose 1996; Denver 2009; Bonett et al. 2010; Voss et al. 2012). Shifts between metamorphosis and paedomorphosis could be simply driven by changes in endogenous hormone levels or hormone sensitivity of target tissues. Mutations are expected to ultimately render nonfunctional any genes and pathways (presumably downstream of thyroid hormone regulation) not being used in paedomorphic lineages, such as those used exclusively during metamorphosis or metamorphic stages. This nonfunctionality is suggested in the obligately paedomorphic salamander families (Amphiumidae, Cryptobranchidae, Proteidae, and Sirenidae) that have exhibited this life history since at least the Cretaceous (Holman 2006; Bonett et al. 2013). These families do not metamorphose, even in the presence of thyroid hormone (Dent 1968; Rose 1996; Safi et al. 2006), suggesting an upper limit on the time during which a regain of metamorphosis could occur. Exogenous thyroxine treatments have been shown to transform individuals of Edwards Plateau *Eurycea* to varying degrees (Kezer 1952; Dundee 1957; Potter and Rabb 1960). However, most thyroid hormone treatments of paedomorphic *Eurycea* were not conducted long enough (or at appropriate concentrations) to determine if metamorphosis could be completed. The maintenance of metamorphosis (complete or near-complete) may suggest at least some essential pathways for metamorphosis are viable, with sufficient hormone concentrations, and may explain the long-term lability of this trait. Natural metamorphosis in *E. troglodytes* is complete (Sweet 1977; R. M.

Bonett, unpubl. obs.), and if this results from a reversal then this shows that the developmental machinery for metamorphosis and terrestrial adult features have been maintained without expression for several million years, and possibly 22 million years.

Alternatively, metamorphosis could have been the ancestral state throughout much of the history of the Edwards Plateau *Eurycea* clade, and paedomorphosis might have independently evolved multiple times. This hypothesis requires many more independent developmental shifts to paedomorphosis as opposed to a single origin and then reversal to metamorphosis, and is significantly less likely from a phylogenetic perspective. However, we do acknowledge that this remains a possibility given the strong ecological associations of paedomorphosis, which could drive this life-history shift among several closely related lineages in the same region.

ECOLOGICAL CORRELATES AND CONSEQUENCES OF PAEDOMORPHOSIS

Our results also shed light on the ecological factors that may drive the evolution of paedomorphosis. Our results support the hypothesis that paedomorphosis may evolve when surface conditions are relatively inhospitable, either because of occurrence in caves (Wake 1966; Ryan and Bruce 2000) or because surface conditions are relatively arid (Sweet 1977). Paedomorphosis may allow for early reproduction and energy conservation (by foregoing metamorphosis) if suitable aquatic conditions are persistent (Semlitsch 1987; Semlitsch et al. 1990; Krenz and Sever 1995; Ryan and Semlitsch 1998). However, paedomorphosis may also be favored if terrestrial habitats are inhospitable. This has been suggested as an explanation for the distribution of metamorphic versus paedomorphic populations of both ambystomatids (Sprules 1974) and plethodontids (Bruce 1976; Bonett and Chippindale 2006). However, this hypothesis has not been tested explicitly with phylogenetic and climatic data. Here, our results from phylogenetic logistic regression strongly support the hypothesis that paedomorphosis evolves in surface-dwelling spelerpines in the more arid parts of the range of the group. A notable exception is that metamorphic populations of *E. troglodytes* are actually in one of the driest areas of the Edwards Plateau (based on annual precipitation; Appendix S1). However, local topography and soil composition in this area create moist canyon slopes, which promote more mesic biotic communities (Sweet 1977; Van Aiken et al. 1981).

We also strongly support the hypothesis that paedomorphosis is associated with the invasion of caves. However, we could not determine which trait transition likely occurred first, or if life history and ecology changed in concert. Subterranean aquifers may offer limited habitat (or resources) for salamanders with terrestrial adult stages, and there are several cases in which evolution of pae-

domorphosis and invasion of caves seem tightly associated (i.e., the three paedomorphic *Gyrinophilus* and *Eurycea wallacei*). Two notable exceptions are *E. spelaea* (cave-dwelling, but not paedomorphic) and the Edwards Plateau *Eurycea*. In Edwards Plateau *Eurycea*, paedomorphosis seemingly evolved prior to invasion of caves, in both the *Typhlomolge* clade and *E. tridentifera*. Furthermore, use of subterranean habitat by nonobligate cave-dwellers occurs repeatedly in the group (e.g., Chippindale et al. 2000). Interestingly, the only other highly specialized cave salamander (*Proteus anguinus*; family Proteidae) is also paedomorphic (Duellman and Trueb 1986). In this case, it appears that paedomorphosis also preceded the invasion of caves, because all proteids are paedomorphic and most *Proteus* are cave-dwellers (Sket and Arntzen 1994; Goricki and Trontelj 2006; AmphibiaWeb 2013). Even for paedomorphic lineages that are not cave-dwelling, such as the paedomorphic populations of *Eurycea tinerensis* in the western Ozarks and some Edwards Plateau species, many populations are seasonally or intermittently subterranean, especially during droughts (Bonett and Chippindale 2006; Bendik and Gluesenkamp 2013). This supports our conclusion that the evolution of paedomorphosis has allowed spelerpines to occur in otherwise inhospitable regions, and possibly invade subterranean adaptive zones (see also Wake 1966; Emel and Bonett 2011).

Our results also highlight some potential disadvantages of paedomorphosis. Previous authors have hypothesized that paedomorphosis may limit dispersal and gene flow among populations (Shaffer 1984). However, recent family-level analyses did not detect significant associations between life history and range characteristics (size or continuity), but trait values were averaged within families (Eastman and Storer 2011). We find that paedomorphic spelerpine plethodontids have significantly smaller geographic range sizes than metamorphosing species, and have made no dispersals between biogeographic regions. We hypothesize that this pattern occurs because paedomorphic spelerpines are largely restricted to cool groundwater springs and headwater streams (Petranka 1998; AmphibiaWeb 2013), and therefore have limited ability to travel overland and increase their range sizes. Reduced range sizes may not be a general property of all paedomorphic salamanders. Specifically, other paedomorphic salamanders of the families Amphiumidae, Cryptobranchidae, Proteidae, and Sirenidae are each broadly distributed and have undergone major dispersal events among or within continents (Milner 1983; Wiens et al. 2005; Bonett et al. 2013). An important difference is that these families all include species that occur in larger aquatic systems such as rivers and coastal flood plains (Petranka 1998), which may facilitate large-scale geographic dispersal. Paedomorphic spelerpines occur in smaller streams, springs, caves, and aquifers that may limit dispersal compared to metamorphs. Overall, our results demonstrate how life-history shifts can influence broad-scale patterns of biogeography.

Finally, we note that both paedomorphic and cave-dwelling lineages have clearly higher speciation rates, but vastly higher extinction rates than metamorphic and surface-dwelling lineages (Table S5). We suspect that this pattern is driven (at least in part) by the small range sizes and/or reduced dispersal ability of paedomorphs and cave-dwellers, which may facilitate allopatric speciation in these lineages and make them more subject to extinction (e.g., Stanley 1986; Cooper et al. 2008; Jablonski 2008; Birand et al. 2012). This phenomenon should be investigated further, but suggests another intriguing macroevolutionary consequence of changes in life-history modes.

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LITERATURE CITED

- AmphibiaWeb. 2013. Information on amphibian biology and conservation. Berkeley, California. Available at <http://amphibiaweb.org/>. Accessed May 22, 2013.
- Bendik, N. F., and A. G. Gluesenkamp. 2013. Body length shrinkage in an endangered amphibian is associated with drought. *J. Zool.* 290:35–41.
- Bendik, N. F., J. M. Meik, A. G. Gluesenkamp, C. R. Roelke, and P. T. Chippindale. 2013. Biogeography, phylogeny, and morphological evolution in central Texas cave and spring salamanders (*Eurycea*). *BMC Evol. Biol.* 13:201.
- Birand, A., A. Vose, and S. Gavrillets. 2012. Patterns of species ranges, speciation, and extinction. *Am. Nat.* 179:1–21.
- Boardman, G. S., and B. W. Schubert. 2011. First Mio-Pliocene salamander fauna from the southern Appalachians. *Palaeontologia Electronica* 14:16A.
- Bonett, R. M., and P. T. Chippindale. 2004. Speciation, phylogeography and evolution of live history and morphology in the salamanders of the *Eurycea multiplicata* complex. *Mol. Ecol.* 13:1189–1203.
- . 2006. Streambed microstructure predicts evolution of development and life history mode in the plethodontid salamander *Eurycea tynerensis*. *BMC Biol.* 4:6.
- Bonett, R. M., E. D. Hoopfer, and R. J. Denver. 2010. Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. *Gen. Comp. Endocr.* 168:209–219.
- Bonett, R. M., A. L. Trujano-Alvarez, M. E. Williams, and E. K. Timpe. 2013. Biogeography and body size shuffling of aquatic salamander communities on a shifting refuge. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 280:20130200.
- Brandley, M. C., J. P. Huelsenbeck, and J. J. Wiens. 2008. Rates and patterns in the evolution of snake-like body form in squamate reptiles: evidence for repeated re-evolution of lost digits and long-term persistence of intermediate body forms. *Evolution* 62:2042–2064.
- Brown, J. M., S. M. Hedtke, A. R. Lemmon, and E. Moriarty Lemmon. 2010. When trees grow too long: investigating the causes of highly inaccurate Bayesian branch length estimates. *Syst. Biol.* 59:145–161.
- Bruce, R. C. 1976. Population structure, life history, and evolution of paedomorphosis in the salamander *Eurycea neotenes*. *Copeia* 1976:242–249.
- Burnham, K. B., and D. Anderson. 2002. Model selection and multi-model inference: a practical information theoretic approach. 2nd ed. Springer-Verlag, New York.
- Camp, C. D., W. E. Peterman, J. R. Milanovich, T. Lamb, J. C. Maerz, and D. B. Wake. 2009. A new genus and species of lungless salamander (family Plethodontidae) from the Appalachian highlands of the southeastern United States. *J. Zool.* 279:86–94.
- Chippindale, P. T., A. H. Price, J. J. Wiens, and D. M. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas hemidactylid plethodontid salamanders. *Herpetol. Monogr.* 14:1–80.
- Chippindale, P. T., R. M. Bonett, A. S. Baldwin, and J. J. Wiens. 2004. Phylogenetic evidence for a major reversal of life-history evolution in plethodontid salamanders. *Evolution* 58:2809–2822.
- Cooper, N., J. Bielby, G. H. Thomas, and A. Purvis. 2008. Macroecology and extinction risk correlates of frogs. *Global Ecol. Biogeogr.* 17:211–221.
- Davis, M. P., P. E. Midford, and W. Maddison. 2013. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. *BMC Evol. Biol.* 13:38.
- Denöel, M., P. Joly, and H. H. Whiteman. 2005. Evolutionary ecology of facultative paedomorphosis in newts and salamanders. *Biol. Rev.* 80:663–671.
- Denver, R. J. 2009. Stress hormones mediate environment–genotype interactions during amphibian development. *Gen. Comp. Endocr.* 164:20–31.
- Dent, J. N. 1968. Survey of amphibian metamorphosis. Pp. 271–311 in W. Etkin and L. I. Gilbert, eds. *Metamorphosis: a problem in developmental biology*. Appleton-century-Crofts, New York.
- Dollo, L. 1893. Les lois de l'évolution. *Bulletin de la Société Belge de Géologie, de Paléontologie et d'Hydrobiologie* 7:164–166.
- Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7:214.
- Duellman, W. E., and L. Trueb. 1986. *Biology of amphibians*. McGraw-Hill, New York.
- Dundee, H. A. 1957. Partial metamorphosis induced in *Typhlomolge rathbuni*. *Copeia* 1957:52–53.
- Eastman, J. M., and A. Storfer. 2011. Correlations of life-history and distributional-range variation with salamander diversification rates: evidence for species selection. *Syst. Biol.* 60:503–518.
- Emel, S. L., and R. M. Bonett. 2011. Considering alternative life history modes and genetic divergence in conservation: a case study of the Oklahoma salamander. *Conser. Genet.* 12:1243–1259.
- Garland, T. Jr., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* 42:265–292.
- Goldberg, E. E., and B. Igic. 2008. On phylogenetic tests of irreversible evolution. *Evolution* 62:2727–2741.
- Goricki, S., and P. Trontelj. 2006. Structure and evolution of the mitochondrial control region and flanking sequences in the European cave salamander *Proteus anguinus*. *Gene* 378:31–41.
- Gould, S. J. 1977. *Ontogeny and phylogeny*. Harvard Univ. Press, Cambridge, MA.
- Heled, J., and A. J. Drummond. 2010. Bayesian inference of species trees from multilocus data. *Mol. Biol. Evol.* 27: 570–580.

- Hijmans, R.J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. The WorldClim Interpolated Global Terrestrial Climate Surfaces, Version 1.3. Available at: <http://www.worldclim.org>. Accessed October 10, 2012.
- Hillis, D. M., D. A. Chamberlain, T. P. Wilcox, and P. T. Chippindale. 2001. A new species of subterranean blind salamander (Plethodontidae: Hemidactyliini: *Eurycea typhlomolge*) from Austin, Texas, and a systematic revision of central Texas paedomorphic salamanders. *Herpetologica* 57:247–410.
- Holman, J. A. 2006. Fossil salamanders of North America. Indiana Univ. Press, Bloomington, IN.
- Huelsenbeck, J. P., and F. R. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- IUCN. 2011. The IUCN Red List of Threatened Species. Version 2011.2. Available at <http://www.iucnredlist.org>. Accessed on August 2011.
- Ives, A. R., and T. Garland. 2010. Phylogenetic logistic regression for binary dependent variables. *Syst. Biol.* 59:9–26.
- Jablonski, D. 2008. Extinction and the spatial dynamics of biodiversity. *Proc. Natl. Acad. Sci. U.S.A.* 105:11528–11535.
- Kezer, J. 1952. Thyroxine-induced metamorphosis of the neotenic salamanders *Eurycea tynerensis* and *Eurycea neotenes*. *Copeia* 1952:234–237.
- Kohlsdorf, T., V. J. Lynch, M. T. Rodrigues, M. C. Brandley, and G. P. Wagner. 2010. Data and data-interpretation in the study of limb evolution: a reply to Galis et al., on the re-evolution of digits in the lizard genus *Bachia*. *Evolution* 64:2477–2485.
- Kozak, K. H., and J. J. Wiens. 2010. Accelerated rates of climatic-niche evolution underlie rapid species diversification. *Ecol. Lett.* 13:1378–1389.
- Kozak, K. H., R. A. Blaine, and A. Larson. 2006. Gene lineages and eastern North American palaeodrainage basins: phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. *Mol. Ecol.* 15:191–207.
- Kozak, K. H., R. W. Mendyk, and J. J. Wiens. 2009. Can parallel diversification occur in sympatry? Repeated patterns of body-size evolution in co-existing clades of North American salamanders. *Evolution* 63:1769–1784.
- Krenz, J. D., and D. M. Sever. 1995. Mating and oviposition in paedomorphic *Ambystoma talpoideum* proceeds the arrival of terrestrial males. *Herpetologica* 51:387–393.
- Lamb, T., and D. A. Beamer. 2012. Digits lost or gained? Evidence for pedal evolution in the dwarf salamander complex (*Eurycea*, Plethodontidae). *PLoS One* 7:e37544.
- Lynch, V. J., and G. P. Wagner. 2010. Did egg-laying boas break Dollo's law? Phylogenetic evidence for reversal to oviparity in sand boas (*Eryx*: Boidae). *Evolution* 64:207–216.
- Maddison, W. P., and D. R. Maddison. 2008. Mesquite: a modular system for evolutionary analysis. Version 2.5. Available at <http://mesquiteproject.org>. Accessed September 30, 2011.
- Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character's effect on speciation and extinction. *Syst. Biol.* 56:701–710.
- Marshall, C. R., E. C. Raff, and R. A. Raff. 1994. Dollo's law and the death and resurrection of genes. *Proc. Natl. Acad. Sci. U.S.A.* 91:12283–12287.
- Milner, A. R. 1983. The biogeography of salamanders in the Mesozoic and early Cenozoic: a cladistic vicariance model. Pp. 431–468 in R. W. Sims, J. H. Price, and P. E. S. Whalley, eds. *Evolution, time and space: the emergence of the biosphere*. Academic Press, Lond.
- Moran, N. A. 1994. Adaptation and constraint in the complex life cycles of animals. *Annu. Rev. Ecol. Syst.* 25:573–600.
- Mueller, R. L. 2006. Evolutionary rates, divergence dates, and the performance of mitochondrial genes in Bayesian phylogenetic analysis. *Syst. Biol.* 55:289–300.
- Mueller, R. L., J. R. Macey, M. Jaekel, D. B. Wake, and J. L. Boore. 2004. Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proc. Natl. Acad. Sci. U.S.A.* 101:13820–13825.
- Niemiller, M. L., B. M. Fitzpatrick, and B. T. Miller. 2008. Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene genealogies. *Mol. Ecol.* 17:2258–2275.
- Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden.
- Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* 255:37–45.
- Pagel, M. and A. Meade. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am. Nat.* 167:808–825.
- Pagel, M., A. Meade, and D. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* 53:673–684.
- Petranka, J. W. 1998. Salamanders of the U. S. and Canada. Smithsonian Institution Press, Washington, DC.
- Potter, F. E., and G. B. Rabb. 1960. Thyroxin induced metamorphosis in a neotenic salamander, *Eurycea nana* Bishop. *Zool. Ser. A. & M. College of Texas* 1:1–12.
- Pyrón, R. A., and J. J. Wiens. 2011. A large-scale phylogeny of Amphibia including over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol. Phylogenet. Evol.* 61:543–583.
- Rambaut, A., and A. J. Drummond. 2007. Tracer v1.5. Available at <http://beast.bio.ed.ac.uk/Tracer>. Accessed June 30, 2012.
- Ree, R. H., and S. A. Smith. 2008. Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57:4–14.
- Ree, R. H., B. R. Moore, C. O. Webb, and M. J. Donoghue. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* 59:2299–2311.
- Revell, L. J. 2012. Phytools: phylogenetic tools for comparative biology (and other things). Available at <http://faculty.umb.edu/liam.revell/phytools/>. Accessed September 18, 2012.
- Roelants, K., D. J. Gower, M. Wilkinson, S. P. Loader, S. D. Biju, K. Guillaume, L. Moriau, and F. Bossuyt. 2007. Global patterns of diversification in the history of modern amphibians. *Proc. Natl. Acad. Sci. USA* 104:887–892.
- Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- Rose, C. S. 1996. An endocrine-based model for developmental and morphogenetic diversification in metamorphic and paedomorphic urodeles. *J. Zool.* 239:253–84.
- Ryan, T. J., and R. C. Bruce. 2000. Life history evolution and adaptive radiation of hemidactyliine salamanders. Pp. 303–325 in R. C. Bruce, R. G. Jaeger, and L. D. Houck, eds. *The biology of plethodontid salamanders*. Kluwer Academic, Plenum Publishers, New York.
- Ryan, T. J., and R. D. Semlitsch. 1998. Intraspecific heterochrony and life history evolution: decoupling somatic and sexual development in a facultatively paedomorphic salamander. *Proc. Natl. Acad. Sci. USA* 95:5643–5648.
- Safi, R., V. Vlaeminck-Guillem, M. Duffrais, I. Seugnet, M. Plateroti, A. Margotat, M. Duterque-Coquillaud, E. J. Crespi, R. J. Denver, B. Demeneix, et al. 2006. Paedomorphosis revisited: thyroid hormone receptors are functional in *Necturus maculosus*. *Evol. Dev.* 8:284–292.

- Semlitsch, R. D. 1987. Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. *Ecology* 68:994–1002.
- Semlitsch, R. D., R. N. Harris, and H. M. Wilbur. 1990. Paedomorphosis in *Ambystoma talpoideum*: maintenance of population variation and alternate life-history pathways. *Evolution* 44:1604–1613.
- Shaffer, H. B. 1984. Evolution in a paedomorphic lineage. I. An electrophoretic analysis of Mexican ambystomatid salamanders. *Evolution* 38:1194–1206.
- Shaffer, H. B., and S. R. Voss. 1996. Phylogenetic and mechanistic analysis of a developmentally integrated character complex: alternate life history modes in ambystomatid salamanders. *Amer. Zool.* 36:24–35.
- Sket, B., and J. W. Arntzen. 1994. A black, non-trogomorphic amphibian from the karst of Slovenia: *Proteus anguinus parkelj* n. ssp. (Urodela: Proteidae). *Bijdr. Dierk.* 64:33–53.
- Sprules, W. G. 1974. The adaptive significance of paedogenesis in North American species of *Ambystoma* (Amphibia: Caudata): an hypothesis. *Can. J. Zool.* 52:393–400.
- Stanley, S. M. 1986. Population-size, extinction, and speciation: the fission effect in Neogene Bivalvia. *Paleobiology* 12:89–110.
- Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, New York.
- Sweet, S. S. 1977. Natural metamorphosis in *Eurycea neotenes*, and the generic allocation of the Texas *Eurycea* (Amphibia: Plethodontidae). *Herpetologica* 33:364–375.
- Syme, A. E., and T. H. Oakley. 2012. Dispersal between shallow and abyssal seas and evolutionary loss and regain of compound eyes in cylindroleberidid ostracods: conflicting conclusions from different comparative methods. *Syst. Biol.* 61:314–336.
- Timpe, E. K., S. P. Graham, and R. M. Bonett. 2009. Phylogeography of the Brownback Salamander reveals patterns of local endemism in Southern Appalachian springs. *Mol. Phylogenet. Evol.* 52:368–376.
- Valenzuela, N., and D. C. Adams. 2011. Chromosome number and sex determination coevolve in turtles. *Evolution* 65:1808–1813.
- Van Auken, O. W., A. L. Ford, and J. L. Allen. 1981. An ecological comparison of upland deciduous and evergreen forests of central Texas. *Amer. J. Bot.* 68:1249–1256.
- Vieites, D. R., M. S. Min, and D. B. Wake. 2007. Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proc. Natl. Acad. Sci. U.S.A.* 104:19903–19907.
- Voss, S. R., D. K. Kump, J. A. Walker, H. B. Shaffer, and G. J. Voss. 2012. Thyroid hormone responsive QTL and the evolution of paedomorphic salamanders. *Heredity* 109:293–298.
- Wake, D. B. 1966. Comparative osteology and evolution of the lungless salamanders, Family Plethodontidae. *Mem. So. Cal. Acad.* 4:1–111.
- Weisrock, D. W., H. B. Shaffer, B. L. Storz, S. R. Storz, and S. R. Voss. 2006. Multiple nuclear gene sequences identify phylogenetic species boundaries in the rapidly radiating clade of Mexican ambystomatid salamanders. *Mol. Ecol.* 15: 2489–2503.
- Whiteman, H. H. 1994. Evolution of facultative paedomorphosis in salamanders. *Q. Rev. Biol.* 69:205–221.
- Wiens, J. J. 2007. Global patterns of species richness and diversification in amphibians. *Am. Nat.* 170:S86–S106.
- . 2011. Re-evolution of lost mandibular teeth in frogs after more than 200 million years, and re-evaluating Dollo's law. *Evolution* 65:1283–1296.
- Wiens, J. J., P. T. Chippindale, and D. M. Hillis. 2003. When are phylogenetic analyses misled by convergence? A case study in Texas cave salamanders. *Syst. Biol.* 52:501–514.
- Wiens, J. J., R. M. Bonett, and P. T. Chippindale. 2005. Ontogeny discombobulates phylogeny: paedomorphosis and salamander relationships. *Syst. Biol.* 54:91–110.
- Wiens, J. J., T. N. Engstrom, and P. T. Chippindale. 2006. Rapid diversification, incomplete isolation, and the “speciation clock” in North American salamanders (genus *Plethodon*): testing the hybrid swarm hypothesis of rapid radiation. *Evolution* 60:2585–2603.
- Wilbur, H. M., and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. *Science* 182:1305–1314.
- Zhang, P., and D. B. Wake. 2009. Higher-level salamander relationships and divergence dates inferred from complete mitochondrial genomes. *Mol. Phylogenet. Evol.* 53:492–508.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Climatic data for spelerpine salamander species.

Table S1. Voucher and locality information and GenBank numbers.

Table S2. Primers used to for polymerase chain reactions (PCRs) and sequencing, and PCR cycling and conditions.

Table S3. Models applied to each data partition for Bayesian phylogenetic analyses and divergence time estimation.

Table S4. Life history, habitat, and distributional information.

Table S5. Rate estimates for BiSSE analyses.

Methods S1. Localities used for climate analyses.

Figure S1. Bayesian phylogram of spelerpines based on mitochondrial DNA.

Figure S2. Bayesian phylogram of spelerpines based on *Rag1*.

Figure S3. Maximum likelihood reconstruction of life history.

Figure S4. Maximum likelihood reconstruction of ecology.

References S1. Supplemental references.