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Coincident transitions across elevation and origins of functional innovations drove the phenotypic and ecological diversity of lungless salamanders

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Abstract

Ecological opportunity (EO) is an important catalyst for evolution. Whereas theory often centers around a lineage encountering a source of EO in isolation, in practice they experience numerous sources of opportunity, either concurrently or sequentially. Such multiplicity can obscure the macroevolutionary signature of EO. Here, we test the effects of elevation (a proxy of the "mountain effect") and an array of functional innovations on the evolutionary history of plethodontid salamanders, a diverse and charismatic radiation of lungless amphibians. Functional innovations unlock access to novel microhabitats, ultimately enabling sub-lineages to occupy a diverse range of ecological niches, particularly in lowland areas where those niches are more abundant. Consistent with expanded ecological opportunity, such transitions to lower elevation result in rapid phenotypic evolution. At high elevation, by contrast, rates of phenotypic evolution and phenotypic disparity decline, reflecting a loss of phenotypically extreme ecological specialists. Transitions in elevation and the origin of innovations appear largely coincident among lungless salamanders, suggesting myriad sources of EO. The magnitude of the "mountain effect" on evolutionary rates (~10-fold) is on par or greatly exceeds that of islands, lakes, and coral reefs on other iconic vertebrate radiations. Therefore, we find that elevation acts as a major ecological moderator and, in concert with functional innovations, shapes the ecological and phenotypic diversity of lungless salamanders.

Keywords: ecological opportunity, macroevolution, microhabitat, morphological evolution, mountains, Plethodontidae

Introduction

Ecological opportunity—a surplus of available ecological resources—is widely considered an important catalyst of adaptive radiation (Gillespie et al., 2020; Simpson, 1944; Stroud & Losos, 2016; Yoder et al., 2010). Ecological opportunities may arise from geographic transitions into novel habitats, the origin of a functional innovation, or the extinction of antagonists (Simpson, 1953). Classic examples of important geographic transitions are diverse, including the colonization of islands, lakes, coral reefs, or mountains (Burns et al., 2024; Mahler et al., 2010; Price et al., 2011; Seehausen, 2006; Wake, 1987). Major functional innovations can similarly take many forms (reviewed in Miller et al., 2023); some well-recognized examples include pharyngeal jaws in fishes (Burress et al., 2020; Liem, 1973; Wainwright et al., 2012), adhesive toe pads in anoles and geckos (Burress & Muñoz, 2022; Losos, 2009; Miller & Stroud, 2022), nectar spurs in angiosperms (Fernández-Mazuecos et al., 2019; Hodges & Arnold, 1995), and adhesive silk in spiders (Bond & Opell, 1998). By providing access to novel resources and/or enhancing the functional and ecological repertoire of a lineage, these sources of ecological opportunity are hypothesized to impart distinct macroevolutionary signatures—generally, elevated rates of evolution followed by a period of decline (Glor, 2010; Harmon et al., 2010; Rabosky, 2014; Yoder et al., 2010). Crucially,

however, quantifying the effects of ecological opportunity on macroevolutionary rates is challenging because such opportunities might not evolve in isolation; rather, multiple sources may arise in quick succession, or confluent with other factors (Donoghue & Sanderson, 2015). For example, even when a key innovation has clear and far-reaching functional consequences (such as pharyngeal jaws and adhesive toepads), disentangling their impact on diversification from alternative sources of ecological opportunity, like geographic transitions and other innovations, is challenging (Alencar et al., 2024; Burress & Muñoz, 2022; Roberts-Hugghis et al., 2023; Seehausen, 2006). This multiplicity may obscure the role of any single catalyst of evolutionary change, and likewise our inferences about the macroevolutionary signal of ecological opportunity (Donoghue & Sanderson, 2015; Yoder et al., 2010).

These issues are highly relevant for understanding the evolution of lungless salamanders (Plethodontidae), a phenotypically diverse and species-rich radiation that is widely distributed across elevational gradients, and its constituent species exhibit different types and combinations of functional innovations (Figure 1 and Figure S1). As a clade, lungless salamanders are distributed from near sea level to mountaintops throughout the Appalachians, Sierra Nevada, and Sierra Madre ranges (Figure S1). Decades of work have yielded insights into a diversity of func-
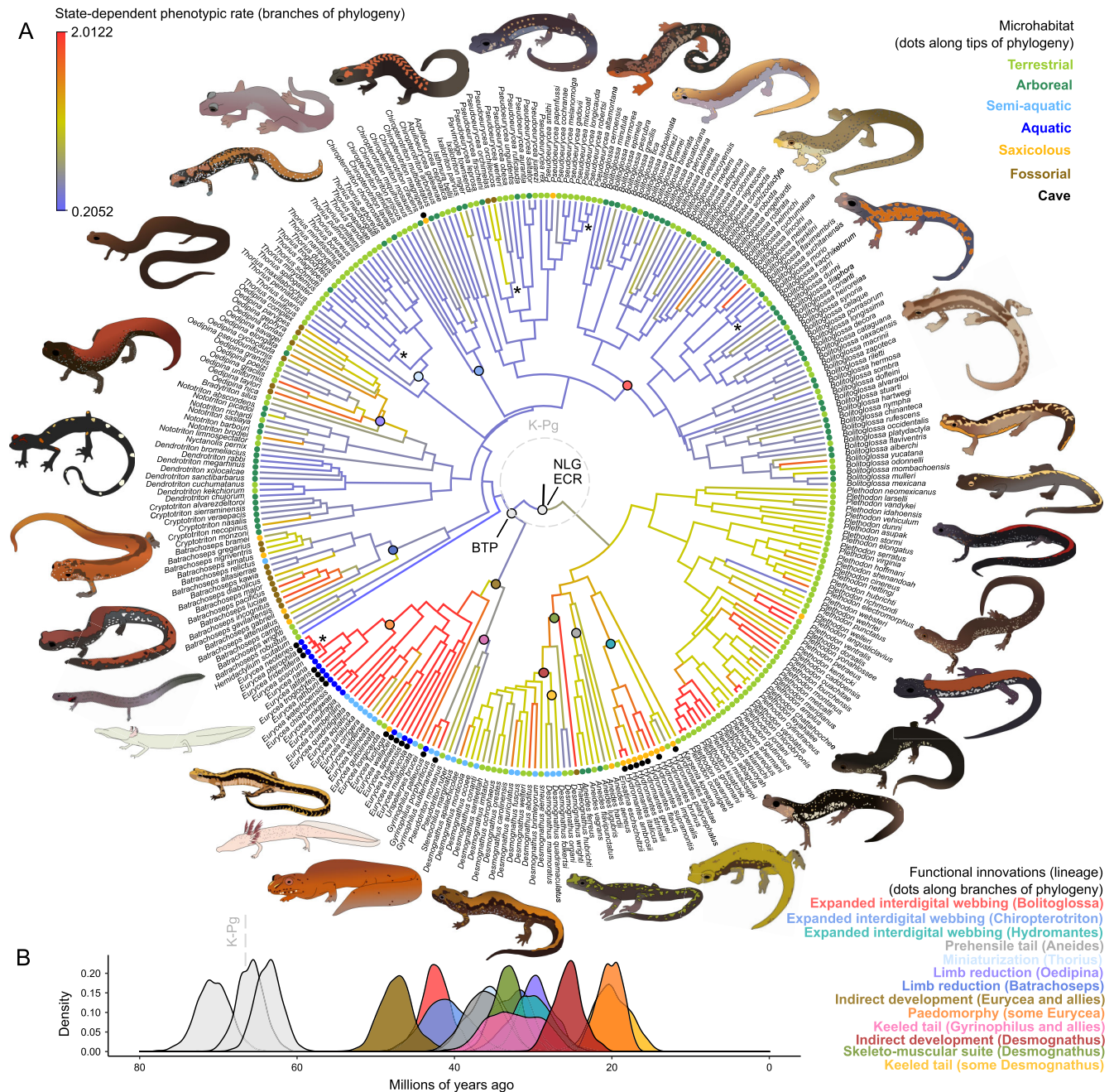
Figure 1. Phylogenetic and temporal origins of functional innovations in lungless salamanders. Branch-specific rates of phenotypic evolution in response to elevation (A). Branch-specific rates are the state-dependent rates weighted by the proportion of time spent in each state. Dots along the tips of the phylogeny indicate the associated microhabitat for each species. Note that six alternative coding schemes were also considered (from Baken & Adams, 2019). Dots along the internal portion of the phylogeny indicate the origins of major functional innovations mentioned in the text (see Supplementary Materials for detailed descriptions). Note that subsequent evolutionary losses of innovations and those that arise along terminal branches are not shown for simplicity. Asterisks denote branches that exhibit shifts to elevated background rates of evolution (i.e., not attributable to elevation; shown in Figure S3). Illustrations by E.D.B. and depict an adjacent species. The ages of functional innovations summarized from 1,000 trees (B). Innovations that arose at or near the root (shown in light gray) are indicated for reference but are not included in the analyses: exclusive cutaneous respiration (ECR), nasolabial groove (NLG), and ballistic tongue projection (BTP).

tional innovations in the lineage (Bonett & Blair, 2017; Parra-Olea & Wake, 2001; Schwenk & Wake, 1993; Wake, 1987, 2009), including prehensile tails and expanded inter-digital webbing that provide an enhanced ability to traverse vertical surfaces, reduced heads and limbs that facilitate fossorial lifestyles, and complex bi-phasic life cycles that relax life history constraints, among other innovations that independently evolved in lungless salamanders (Figure 1).

The evolution of lungless salamanders is further influenced by their sensitivity to environmental conditions, implying an additional role of geographic setting in guiding evolutionary dynamics for species generation and phenotypic diversity (Feder, 1983). These organisms rely on cutaneous respiration, or skin-breathing (Spotila & Berman, 1976) and, consequently, lungless salamanders are generally restricted to cool, moist habitats that minimize ener-

getic demand and maintain moist skin necessary for gas exchange (Baken et al., 2020; Feder, 1983; Feder & Londos, 1984; Kozak & Wiens, 2016; Riddell et al., 2024). Because body size and shape influence rates of water loss in amphibians (Baken et al., 2020; Riddell et al., 2024), mountains—and the array of hydric environments they supply—have the potential to shape morphological diversity via selective pressures to remain hydrated. Therefore, we might expect salamander phenotypic evolution to be driven in part by environmental dimensions, such as elevation, that create steep gradients in temperature and moisture.

Importantly, innovations and mountains could have acted independently or in combination during the evolutionary history of salamanders (Donoghue & Sanderson, 2015). In fact, some salamander functional innovations have been explicitly linked to elevation, including spring-powered tongue-protrusion (i.e., ballistic feeding), which might facilitate expansion into cool montane environments by maintaining higher performance in cool environments than ancestral muscle-powered tongue protrusion would allow (Deban et al., 2020; but see Friedman & Muñoz, 2022). Likewise, expanded interdigital webbing has been proposed to be an innovation tied to widespread use of arboreal microhabitat in tropical montane forests (Wake, 1987). By contrast, other innovations like limb reduction have been linked to transitions from mountains into surrounding lowland areas (Parra-Olea & Wake, 2001). Meanwhile, other innovations, such as adaptations to the skeleton and muscles for digging (i.e., Schwenk & Wake, 1993) lack a clear association with elevation.

In addition to this complex assembly of environmental and phenotypic patterns, plethodontid salamanders are characterized by rate heterogeneity in speciation and phenotypic evolution. Notably, rates of speciation and morphological evolution are uncorrelated in plethodontid salamanders: lineages characterized by rapid morphological change need not also be fast speciating, and vice versa (Adams et al., 2009). For example, several plethodontid genera exhibit a relatively conserved body plan and similar microhabitat ecology despite high species richness and are often considered classic non-adaptive radiations (e.g., the largely terrestrial *Plethodon*; Kozak et al., 2006; Martin & Richards, 2019; Rundell & Price, 2009). Such lineages are characterized by rapid species diversification, often across elevation, despite limited rates of phenotypic evolution (Adams et al., 2009; Baken & Adams, 2019; Blankers et al., 2012). Yet, periods of prolonged morphological stasis in plethodontids have been periodically interrupted by bouts of adaptive radiation as lineages expand into new environments (i.e., lowlands or high elevation; Bruce, 1996; Rovito et al., 2013; Ryan & Bruce, 2000; Wake, 1987; Weaver et al., 2020). This family-wide decoupling between morphological evolution and species accumulation suggests an important role for different sources of ecological opportunity in generating plethodontid diversity. Yet, such heterogeneous ecological, phenotypic, and evolutionary outcomes among lineages have also limited our ability to coalesce different underlying factors into a cohesive hypothesis for the evolutionary history of plethodontid salamanders (Wake, 2009, 2017), urging deeper inquiry into the proposed catalysts of plethodontid diversity.

In this study, we evaluate two prevailing paradigms: (i) the macroevolutionary signature of ecological opportunity (Glor, 2010; Schluter, 2000; Simpson, 1953; Stroud & Losos, 2016) and (ii) that mountains function as a crucible for biodiversity (García-Rodríguez et al., 2021; Noroozi et al., 2018; Vasconcelos et al., 2020). We then unify these ideas with contemporary phylogenetic comparative methods to test three possible scenarios: (i) a scenario in which innovations and changes in elevation separately accelerate salamander morphological evolution (i.e., the origins of innovations and changes in elevation occur at different times and/or parts of the phylogeny), (ii) a scenario in which innovations and changes in elevation occur in conjunction to accelerate salamander morphological evolution (i.e., the origins of innovations and changes in elevation occur simultaneously), and (iii) a scenario in which functional innovations and changes in elevation have no effect on salamander morphological evolution. By stitching together how innovations and elevation interact to shape patterns and rates of morphological evolution in plethodontid salamanders, we provide a more synthetic perspective on the factors shaping their phenotypic diversity and contextualize the mechanisms decoupling phenotypic evolution and speciation in this highly diverse lineage of amphibians.

Materials and methods

Species sampling and phylogenetic tree

We used an existing time calibrated phylogeny for lungless salamanders from Bonett & Blair (2017). This phylogeny included 516 species across Caudata and was estimated from three mitochondrial and four nuclear genes. For phylogenetic comparative analyses, we pruned the maximum clade credibility tree to match the sampling in our dataset (306 species; see below). This sampling includes 27 genera and species from the Appalachian, Ozark, Sierra Nevada, and Sierra Madre mountains and spans North, Central, and South America.

Phenotypic traits

We used an existing dataset of morphological traits from Baken & Adams (2019), which was based on 1–33 specimens (mean = 10.2) per species. Since within-species sexual dimorphism is small compared to between species differences (Petranka, 1998), sexes were combined, as in other studies (e.g., Blankers et al., 2012). The traits were linear distances that capture a large proportion of the variation in salamander body shape (Adams et al., 2009; Bonett & Blair, 2017). Morphological measurements include traits commonly associated with microhabitat use (originally from Adams et al., 2009; Wiens & Hoverman, 2008): snout-to-vent length (SVL), snout length, head length, body width, forelimb length, hindlimb length, and tail length. Prior to phylogenetic comparative methods, we calculated phylogenetic residuals by regressing each ln-transformed trait against ln-transformed SVL with the phyl.resid function implemented in the *phytools* R package (Revell, 2012).

Functional innovations

We summarized the origination of 13 proposed functional innovations across the plethodontid phylogeny and through

time. These innovations include expanded interdigital webbing (in *Bolitoglossa*, *Chiropterotriton*, and *Hydromantes*), prehensile tails in *Aneides*, miniaturization in *Thorius*, limb reduction (in *Oedipina* and *Batrachoseps*), indirect development (in *Eurycea* and allies as well as in some *Desmognathus*), paedomorphy in some *Eurycea*, keeled tails (in some *Desmognathus* as well as in *Gyrinophilus* and allies), and the skeletomuscular suite in *Desmognathus*. The point of origination (i.e., branch on the phylogeny) for each innovation was based on the literature (see Supplementary Materials for descriptions of each innovation, their inferred adaptive feature, and associated references). Whether a certain trait should be considered an innovation varies according on one's definition (reviewed in Miller et al., 2023); for the purpose of this study, we considered all proposed innovations equally and tested whether phenotypic evolution accelerates following their origination. Using these focal clades, we visualized the temporal pattern of origination by extracting node ages from 1,000 trees sampled from the posterior distribution (originally from Bonett & Blair, 2017).

Elevation data

We obtained occurrence records for 306 species representing 28 plethodontid genera from the Global Biodiversity Information Facility (GBIF) using the *rgbif* package in R (Chamberlain et al., 2021). We cleaned the resulting GBIF records to exclude those lacking coordinates or with coordinate uncertainty >50 km (due to coordinate precision), duplicate records, and other erroneous records, including those located in oceans or unduly geographically isolated using the package *CoordinateCleaner* (Zizka et al., 2019). The final elevation dataset ranged from 1 to 754 unique observations (mean = 104) per species. Most species with a single unique observation were highly restricted in their ranges, such as cave or montane endemics. We extracted elevation for each occurrence record from the Shuttle Radar Topography Mission (SRTM) digital elevation layer at a 1 arc-second resolution (~30 m) (Farr et al., 2007) using Google Earth Engine. The SRTM Digital Elevation Model dataset was selected as it is the finest scale elevation data covering the extent of the occurrence records.

Rates of phenotypic evolution

Quantifying the effect of elevation on rates of phenotypic evolution requires isolating its signature from that of other factors that can elicit rate shifts in salamanders (e.g., Baken & Adams, 2019; Blankers et al., 2012; Bonett & Blair, 2017; Ledbetter & Bonett, 2019). Therefore, it was important to account in our analyses for alternative sources of rate heterogeneity beyond elevation. To this end, we estimated state-dependent rates of phenotypic evolution using MuSSCRat (May & Moore, 2020) employed in RevBayes (Höhna et al., 2016). This method accounts for background rate variation (i.e., rate variation not attributable to the character of interest (elevation, in this case), simultaneously estimates the evolutionary histories of the discrete and continuous characters, and uses a multivariate framework to account for shared evolutionary histories of continuous characters (May & Moore, 2020). For the discrete character, we used four elevation quartiles: lowland (0–640 m), midlow (641–1069 m), midhigh (1070–1689 m), and highland (1690–3326 m; Figure S2). For continuous characters, we included

phylogenetic residuals for snout length, head length, body width, forelimb length, hindlimb length, and tail length. The Markov chain Monte Carlo (MCMC) was run for 100,000 generations with 10% burn-in. The model estimates state-dependent rates (for each elevation quartile), branch-specific rates (along every branch), and rates for each phenotypic trait. To test for sensitivity to priors, we repeated analyses across different prior numbers of rate shifts (means of 1, 25, 50, 75, and 100 shifts with lognormal distributions). To further evaluate the robustness of results, we repeated MuSS-CRat using two alternative models of evolution—a random local clock in which the rates of descending branches are inherited based on a probability of a rate shift (i.e., rates have phylogenetic structure) and an uncorrelated lognormal model in which rates do not have phylogenetic structure (Burress & Muñoz, 2022; May & Moore, 2020). Further, to evaluate sensitivity to cut-offs used to discretize elevation (quartiles) and owing to their subjectivity, we performed two additional runs using alternative cut-offs to define states for the discrete character—a model with two states (upper and lower 50th percentiles; lowlands and highlands) and a model with three states (lower, mid, and upper 33rd percentiles; lowlands, intermediate, and highlands; Figure S2).

Next, to test whether the origins of functional innovations were associated with evolutionary rate shifts, we considered shifts in rates in response to elevation (i.e., state-dependent rates) as well as shifts in the background rates (i.e., in response to factors other than elevation). Although the state-dependent rates are driven by elevation (not innovations), the tendency for functional innovations to arise in association with transitions to higher/lower elevation (Parra-Olea & Wake, 2001; Rovito et al., 2013; Wake, 1987) will let us assess the coincident nature of these separate features. A major issue that extends from rate heterogeneity is that there are multiple configurations of rate shifts that can explain rate variation across the phylogeny (Louca & Pennell, 2020; Moore et al., 2016; Rabosky, 2014). Therefore, rather than interpreting the best supported configuration of rate shifts or setting an arbitrary threshold for interpreting rate shifts as significant, we instead assessed if rate shifts were disproportionally concentrated around nodes at which functional innovations arose using the posterior probability of rate shifts along all branches. This approach characterizes rate shifts as proportional to their occurrence in the posterior distribution. We assessed two types of rate shifts based on their proximity to the origin of a functional innovation (illustrated in Figure S3). Because the origins of innovations were estimated at focal nodes (e.g., often as a synapomorphy of the focal clade) whereas rate shifts were estimated along branches, we considered rate shifts along the leading branch (i.e., immediately rootward) as being *coincident* rate shifts since the innovation would have naturally arisen along this branch (not at the focal node; Figure S3; Burress & Muñoz, 2023). Since macroevolutionary responses such as changes in evolutionary rates can involve a degree of lag time, we also considered shifts up to two descending (i.e., tipward) branches from the focal node (i.e., Burress & Muñoz, 2023; Burress & Tan, 2017; Figure S3). These are referred to as *delayed* rate shifts. Owing to the necessity of sufficient branches and branch length to properly estimate branch rates, and subsequently, the locations of rate shifts, we only interpret rate shifts for innovations at nodes at least 20% of tree length away from the root (i.e., ex-

cluding innovations that arose at or near the root, such as ballistic tongue projection or exclusive cutaneous respiration). Likewise, we exclude innovations that arose along terminal branches (e.g., indirect development in *Hemidactylum scutatum*) since there are no downstream branches along which to assess rate shifts or microhabitat transitions.

We tested for "elevation effects" on the rate of phenotypic evolution in two additional ways in which elevation was treated as a continuous character. First, we employed the rate-by-state test (modified from Reynolds et al., 2016). During this procedure, multivariate contrasts (McPeek et al., 2008) were regressed against the elevation midpoint ancestral states. Phylogenetic independent contrasts were calculated with the *pic()* function in the *ape* R package (Paradis & Schliep, 2019). Ancestral states were estimated with the *fastAnc()* function in *phytools* (Revell, 2012). We next employed a further modified rate-by-state test in which tip rates were regressed against elevation midpoint. Tip rates are an estimate of contemporary diversification conditioned on evolutionary history (Title & Rabosky, 2019), whereas the other methods, MuSSCRat and multivariate contrasts, are informed by all depths of the tree (i.e., rates along all branches or point estimates at all nodes, respectively). We estimated tip rates of phenotypic evolution using a multivariate relaxed local clock model employed in RevBayes (Burress et al., 2020; Burress & Muñoz, 2022; May & Moore, 2020). Since tip rates are not independent, we assessed the correlation between tip rates and elevation midpoint using phylogenetic generalized least squares (Revell, 2010). Since limb reduction is treated as an innovation and limb length is a trait included in the study (one of six that describe variation in body shape), we assessed the potential for statistical circularity when associating the origins of innovations with rate shifts using the rate-by-state test as described above. The rate of limb evolution was uncorrelated with limb length (Figure S4), indicating statistical independence between rate and state.

To assess the possibility that our degree of taxon sampling could lead to false positives (i.e., type-1 error), we simulated 100 datasets comprised of six traits (to match the empirical dataset) across the Plethodontidae phylogeny using single-rate BM with the *sim.char()* function employed in *geiger* (Harmon et al., 2008). During this procedure, we incorporated the empirical covariance among traits and used the pairwise trait correlations estimated from the multivariate evolutionary rate analyses to generate corresponding simulated multivariate datasets. Since these correlations are estimated dynamically alongside the evolutionary rates, the values varied slightly across runs with different priors (Figure S5); we arbitrarily used the mean values from the model with a prior of 100 rate shifts during the simulation procedure. We then randomly pruned the simulated datasets to 62% taxon sampling to match the empirical degree of taxon sampling. We then repeated the MuSSCRat and rate-by-state analyses using these simulated datasets. All settings were as described above, except that the MCMCs were stopped when Effective Sample Size > 200 to facilitate completion of the 100 runs.

Microhabitat data
Above, we described how we estimated rates of phenotypic evolution across elevation; to help contextual-

ize these results, we were next interested in how rates across elevation reflected the relative use of different microhabitats. To this end, we classified species based on adult microhabitat use (modified from Baken & Adams, 2019), which was based on a combination of published literature and field observations (Blankers et al., 2012; McEntire, 2016; Petranka, 1998; Wake, 1987; Wake & Lynch, 1976). Microhabitat categories included terrestrial, arboreal, saxicolous, semi-aquatic, aquatic, fossorial, and cave (see Supplementary Materials for descriptions, classification rationale, and discussion of uncertainty in microhabitat classification). We further refined salamander microhabitat classifications through direct feedback from eight experts in the plethodontid research community (listed in Supplementary Materials and in the Acknowledgements below). We estimated the stochastic history of microhabitat use with *corHMM* (Boyko & Beaulieu, 2021) using several transition models in which transition rates were equal (ER), transition rates between state pairs were equal but could vary between state pairs (SYM), and all transition rates were different (ARD). Additionally, we ran a second version of each model with two rate categories (Boyko & Beaulieu, 2021). The root state was set to "terrestrial" following prior studies across broader phylogenetic scales (Baken & Adams, 2019). The "cave" character state was treated as polymorphic such that it was an alternative state for some aquatic, semi-aquatic, and saxicolous species (Baken & Adams, 2019; Figure 1). Owing to the subjectivity of microhabitat classifications (Baken & Adams, 2019) and a lack of biological justification, we did not prohibit any types of transitions. We summarized and plotted the output of these models using *corHMM* (Boyko & Beaulieu, 2021) and *phytools* (Revell, 2012), respectively. We then summarized the diversity of microhabitat specialists across elevation. For each elevation quartile, we calculated microhabitat diversity (i.e., the number of microhabitat specialists present). Since there is some inherent conflict when designating a microhabitat for some species (e.g., some species are aquatic *and* live in caves), we summarized the number of specialists present within each quartile across all six alternative microhabitat coding schemes presented by Baken & Adams (2019). We note that none of the rate (MuSSCRat or rate-by-state tests) or disparity analyses in the main text are informed a priori by the microhabitat classifications. To assess if the proportion of branches associated with innovations (13 branches) that also have microhabitat transitions (7 of 13 branches) was remarkable or could be explained by random chance, we randomly selected 13 branches and assessed how many had microhabitat transitions and repeated this procedure 1,000 times to generate a null expectation for this proportion (i.e., Maddison, 1990). Since we excluded the terminal branches during the empirical analyses, we also excluded them during the calculations of the null expectation. Although these random branches were otherwise sampled proportionally to their representation in the phylogeny, we directly assessed the length of branches along which innovations arose in case they disproportionately arose on long branches.

Phenotypic disparity and diversity
For each elevation quartile, we calculated phenotypic disparity and performed pairwise comparisons using the *morphol.disparity* function implemented in the *geomorph* R
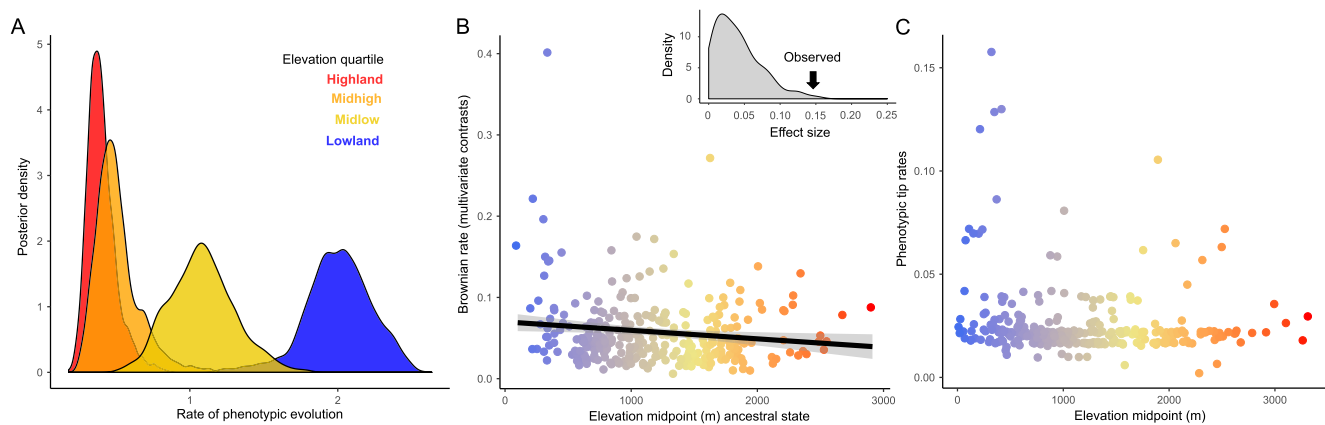
Figure 2. Rates of phenotypic evolution in response to elevation in lungless salamanders. State-dependent rates across elevation quartiles (A), multivariate contrasts regressed against the elevation ancestral state (B), and tip rates regressed against elevation (C). The inset density depicts the null distribution of effect sizes and the observed effect size.

package (Adams & Otárola-Castillo, 2013) using 999 iterations. During this procedure, disparity is measured as the variance, and the calculations were performed in a phylogenetic context (e.g., using procD.pgls as input).

Results

Lungless salamanders occupy an exceptionally wide elevational range, occurring from sea level (e.g., many *Eurycea* and *Plethodon*) to nearly 5,000 meters above it (e.g., *Aquiloeurycea cephalica*, *Pseudoeurycea leprosa*, and *Thorius dubitus*), with elevational midpoints ranging from just above sea level to over 3,300 m (Figures S1 and S2). Species richness was biased toward lower elevations, with a modest species-richness hump at mid-elevation (i.e., 2,000–3,000 m; Figure S1). Functional innovations were widely distributed across the radiation, often arose independently (i.e., multiple origins of the same innovation), but rarely in immediate proximity to other innovations (i.e., at adjacent nodes), except near the root and within *Desmognathus* (Figure 1). Isolating the effect of elevation on branch-specific phenotypic evolution revealed that rates varied by an order of magnitude (9.8-fold) (Figure 1). Rates of phenotypic evolution were dependent on elevation (PP = 1.0) and decreased 4.5-fold, on average, between the lowland and highland quartiles (Figure 2A). These results were consistent using different priors and evolutionary models (Figures S6 and S7), as well as different numbers of states for the discrete character (Figure S8). Background rate heterogeneity (i.e., evolutionary rate variation not attributable to elevation) included isolated bouts of elevated rates in *Eurycea*, *Gyrinophilus*, *Thorius*, *Pseudoeurycea*, and *Bolitoglossa*, and varied up to 68-fold (Figure S9). Traits closely associated with adaptation for microhabitat use (i.e., limb and tail length) evolved faster than other traits (i.e., head dimensions; Figure S10). The rate of phenotypic evolution, as estimated with multivariate contrasts, was negatively correlated with elevation using the rate-by-state test ($r = 0.139$, $f = 5.97$, $p = .010$; Figure 2B); tip rates, however, were not correlated with elevation ($r = 0.001$, $t = 0.117$, $p = .907$; Figure 2C). The correlation between multivariate contrasts and elevation was also significant if data beyond the 95th Confidence Interval were

excluded as outliers ($p < .05$), indicating that the result was not driven by a few extreme data points. Analyses with BM-simulated datasets did not elicit type-1 errors, as rates of evolution were not state-dependent (all PP < 0.14) or correlated with elevation (all $p > .675$). These results suggest that our degree of taxon sampling is unlikely to produce false positives. In 10 of 13 cases, the origin of functional innovations was associated with shifts in the rate of phenotypic evolution (Table 1; Figure 3; Figure S11). This association between the origin of functional innovations and rate shifts was consistent across different priors on the number of rate shifts (Figure S12A, B). Of the 10 functional innovations that were associated with rate shifts, nine were shifts in rates as they varied in response to elevation: specifically, transitions to lower elevation, faster phenotypic rates, and the origins of functional innovations generally occurred along the same branches of the phylogeny (Figures 1, 3, Figures S11, S12). By contrast, only one innovation was associated with shifts in background evolutionary rates (within *Thorius*; Figure S9).

In addition to rates of phenotypic evolution, the diversity of microhabitat specialists also generally declined with elevation across all microhabitat coding schemes (Figure S13). Specifically, the relative proportion of terrestrial and arboreal salamanders generally increased with elevation, and all other microhabitats declined (Figure 4A). At high elevation, saxicolous, semi-aquatic, aquatic, fossorial, and cave salamanders were altogether absent (Figure 4A). This pattern had a profound effect on phenotypic diversity across the elevational gradient, as ecological specialists occupying phenotypic extremes, like cave specialists, were absent at high elevation (Figure S14). Consequently, salamander phenotypic disparity conspicuously declined at high elevation (Figure 4B and Figure S13). The evolutionary history of microhabitat use best fits a single rate symmetrical transition model (Table S1). The most frequent transitions were between terrestrial and arboreal states (Figure S15A, B). Transitions in microhabitat use were associated with functional innovations in six of 13 cases (46%; Table 1). This proportion (0.46) exceeded random expectation (0.16–0.21) and was consistent across all alternative microhabitat coding schemes (range: 0.16–0.31). Further, we confirmed that innovations did not arise along particularly long branches, as none of the 13 in-

Table 1. Functional innovations and their associated shifts in microhabitat use and rates of phenotypic evolution during the evolutionary history of lungless salamanders.

Innovation	Lineage	Microhabitat transition	Phenotypic rate shift	Prop.
EIW	*Bolitoglossa*	No	No	N/A
EIW	*Chiropterotriton*	Yes[Arboreal]	No	N/A
EIW	*Hydromantes*	Yes[Saxilocous]	Yes[coin,incr]	0.50
PT	*Aneides*	No	Yes[coin,incr]	0.75
SMS	Desmognathines	No	Yes[coin,incr]	0.50
IDD	*Desmognathus* (in part)	No	Yes[coin,decr]	1.00
KT	*Desmognathus* (in part)	Yes[Semiaquatic]	Yes[del,decr]	1.00
IDD	Hemidactylini	Yes[Semiaquatic]	Yes[del,incr]	0.50
PAE	Some *Eurycea*	Yes[Aquatic]	No	N/A
KT	*Gyrinophilus* and allies	No	Yes[del,incr]	0.33
LR	*Batrachoseps*	Yes[Fossorial]	Yes[coin,incr]	0.25
LR	*Oedipina*	No	Yes[coin,incr]	0.75
MIN	*Thorius*	No	Yes[del,incr]	0.50

See Figure 1 and Figure S10 for the location and support for rate shifts. For each Innovation (defined in footnote), we provide the corresponding Lineage, whether the innovation is associated with a Microhabitat transition and a Phenotypic rate shift (see footnote), and the proportion of descendant lineages that exhibit the associated rate change (Prop.).
Footnote: The timing and direction of rate shifts are denoted with superscript: coincident with the origin of the innovation (coin), delayed following the origin of the innovation (del), rate increase (incr), rate decrease (decr). Innovations: indirect development (IDD), paedomorphy (PAE), prehensile tail (PT), expanded interdigital webbing (EIW), skeletomuscular suite (SMS), keeled tail (KT), limb reduction (LR), and miniaturization (MIN).
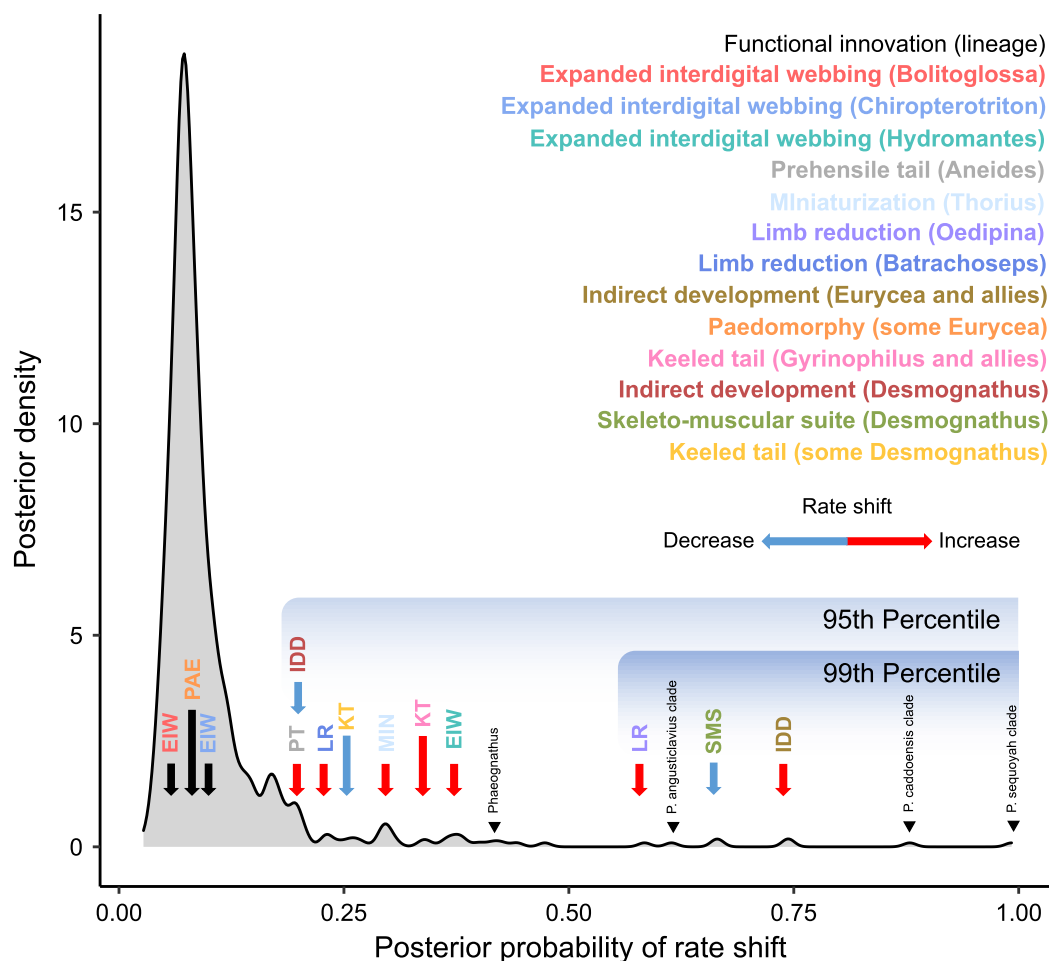

Figure 3. Rate shifts are disproportionately associated with the origins of functional innovations. The shaded area is the posterior probabilities of rate shifts along all branches. Labelled arrows highlight branches associated with the origin of a functional innovation. Triangles indicate additional clades associated with rate shifts. Data used from the random local clock model with a prior of 50 rate shifts (see Figures S6 and S7 for comparisons of different models).
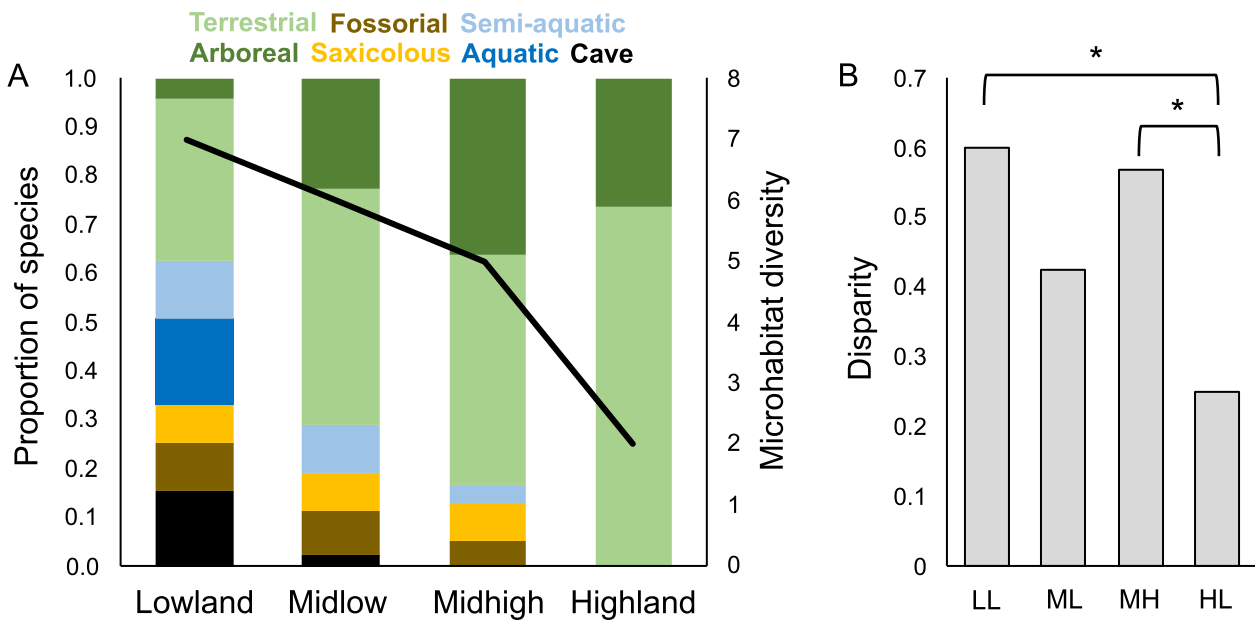
Figure 4. Microhabitat diversity of lungless salamanders across the elevational gradient. Proportion of species (A) and phenotypic disparity (B) across elevation quartiles. Asterisks denote significantly different disparity ($p < .05$).

novations were associated with branches in the 90th percentile of branch length, and only two were in the 75th percentile of branch lengths. On average, branches associated with the origin of a functional innovation were 2.5 times more likely to have a transition in microhabitat than were randomly selected branches. Further, lineages in which functional innovations evolved also tended to have different phenotypes, varying in the relative length of the limbs and tail and width of the trunk ([Figure 5A]). The functional innovations were also well distributed across the elevational gradient ([Figure 5B]).

Discussion

Ecological opportunity is a renowned trigger for biodiversity, catalyzing both speciation and phenotypic evolution, two critical features of adaptive radiation ([Schluter, 2000]; [Seehausen, 2006]; [Simpson, 1953]). Whether by release from antagonists or by origination of a key innovation, opportunity opens access to new ecological space, prompting adaptive evolution and speciation. Yet, opportunities are unlikely to arise in isolation; across epochal timescales, heterogeneous mixture of opportunities will yield complex macroevolutionary patterns and rates that are difficult to disentangle ([Alencar et al., 2024]; [Donoghue & Sanderson, 2015]). These macroevolutionary disparities are manifest in plethodontid (lungless) salamanders, a diverse radiation characterized by heterogeneous rates of morphological evolution and speciation ([Adams et al., 2009]). Here, we isolated and quantified the effects of elevational shifts and key innovations on salamander diversity. We found that different sources of ecological opportunity operate in concert to shape the rate and pattern of phenotypic evolution in lungless salamanders. We show that transitions to lower elevation are robustly associated with accelerated phenotypic evolution in lungless salamanders ([Figures 2] and [Figures S6–

S8]; [Table 1]), and that these effects coincide with the origins of functional innovations, and subsequently, with changes in microhabitat use. We unpack these findings in greater detail below and describe how different sources of opportunity assembled to shape the remarkable diversity of lungless salamanders.

Functional innovations as keys to unlock access to ecological opportunity

By providing evolutionary access to novel ecological resources, functional innovations are a canonical source of ecological opportunity ([Schluter, 2000]; [Simpson, 1953]; [Stroud & Losos, 2016]; [Yoder et al., 2010]). Although lungless salamanders exhibit a broad range of evolutionary outcomes, including both adaptive radiations ([Bruce, 1996]; [Rovito et al., 2013]; [Wake, 1987]; [Weaver et al., 2020]) and classic non-adaptive radiations ([Kozak et al., 2006]; [Martin & Richards, 2019]; [Rundell & Price, 2009]), we can coalesce these varied outcomes by evaluating the signatures of functional innovations and ecological opportunity on morphological evolution across elevation. Functional innovations arose coincident with (or immediately prior to) all the major lineages posed as adaptive radiations in the literature—*Bolitoglossa* (expanded interdigital webbing), *Thorius* (miniaturization), *Oedipina* (reduced limbs and head), *Desmognathus* (adaptations for burrowing), and the hemidactylines (spring-powered tongue-projection and indirect development; [Bruce, 1996]; [Parra-Olea & Wake, 2001]; [Rovito et al., 2013]; [Schwenk & Wake, 1993]; [Wake, 1987]; [Weaver et al., 2020]). Functional innovations nearly always coincide with transitions in elevation and microhabitat use ([Table 1]; [Figure 1]), consistent with macroevolutionary signatures of ecological opportunity, namely innovations unlocking access to novel environmental space and ecological niches ([Glor, 2010]; [Simpson, 1944]; [1953]; [Stroud & Losos, 2016]; [Yoder et al., 2010]). By contrast, prolonged pe-
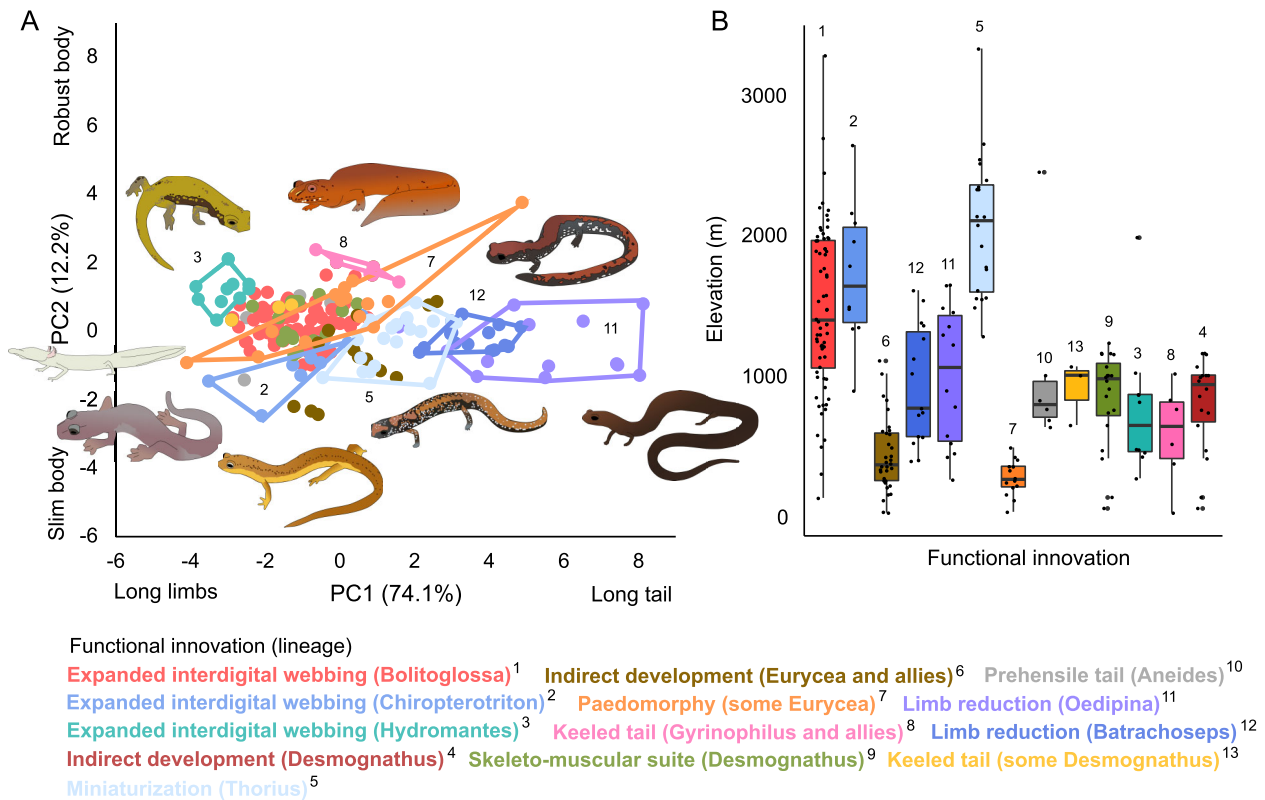
Figure 5. Phenotypic diversity of lungless salamanders (A). Innovation-bearing lineages are indicated in color. Select lineages are further highlighted with minimum convex hulls. The distribution of innovation-bearing lineages across the elevational gradient (B). Dots in both panels are species means. Inset numbers complement colors to help associate the groups. Illustrations depict an adjacent species. Illustrations by E.D.B.

riods of ecological stasis observed in groups often viewed as non-adaptive radiations (e.g., *Plethodon*; Kozak et al., 2006; Martin & Richards, 2019; Rundell & Price, 2009) conspicuously lack major functional innovations (at least near their origin) and, consequently, exhibit comparatively constant rates of phenotypic evolution and fewer transitions in elevation and microhabitat use (Figures 1, Figures S2, and S15). Nevertheless, there are high rates of phenotypic evolution in several *Plethodon* sub-lineages, including multiple clades of slimy salamanders (Figures 1 and 3). Species in this lineage also vary in fine-scale microclimatic habitat use, suggesting physiological diversity might be high despite limited ecomorphological variation (Farallo et al., 2020), a possibility inviting further inquiry (Wake, 2009; Wake et al., 1983).

Dusky salamanders (*Desmognathus*) stand out as having three functional innovations that arose in close phylogenetic proximity (i.e., at nearly adjacent nodes; Figure 1). All three innovations reflect an increasing association with aquatic habitat—adaptations for feeding and burrowing (SMS), indirect development, and keeled tails (Bonett & Blair, 2017; Bruce, 1996; Schwenk & Wake, 1993; Weaver et al., 2020). After an initial increase in phenotypic evolution associated with the SMS innovation, both subsequent innovations were associated with shifts to slower rates of phenotypic evolution (Table 1). This may reflect their rapid occupation of an adaptive peak associated with stream margins and seepage (Kozak et al., 2005; Weaver et al., 2020) and subsequent decline in evolutionary rates as that niche filled (i.e., Freckleton & Harvey, 2006). This lineage is also experiencing a recent

surge in their recognized species diversity (Pyron & Beamer, 2022a; b; Pyron et al., 2022), making it a fruitful group for further inquiry.

Although paedomorphy is associated with accelerated phenotypic evolution among several lineages of Caudata (Bonett & Blair, 2017; Fabre et al., 2020), we are unable to corroborate this notion within Plethodontidae. There was a well-supported, high-magnitude rate shift at the base of the clade comprised of *Eurycea* and *Urspelerpes* associated with a transition to low elevation (Figure 1), then a subsequent rate shift in background rates associated with the clade comprised of *E. neotenes*, *E. pterophila*, *E. tridentifera*, and *E. sosorum* (Figure S8). These two rate shifts are significantly preceding and subsequent to the origin of paedomorphy, respectively (Figure 1), perhaps highlighting the importance of accounting for background rate variation when interpreting catalysts of elevated rates of phenotypic evolution (Burress & Muñoz, 2022; May & Moore, 2020). Additional functional innovations may come to light in the future, as our understanding of plethodontid species diversity and biology expands. Likewise, our understanding of currently recognized functional innovations may change. For example, the Atlantic Coast slimy salamander, *Plethodon chlorobryonis*, is known to have a prehensile tail (Mittleman, 1951), but how common this feature is among other slimy salamanders is unclear. This species exhibits elevated rates of phenotypic evolution (Figure 1), so additional knowledge about the distribution of prehensile tails in slimy salamanders could better contextualize some rate shifts within *Plethodon* (Figure 3).

The "mountain effect" and shifting physiological constraints

Mountains are historically viewed as biodiversity engines, often characterized by high species richness in high-elevation areas (García-Rodríguez et al., 2021; Noroozi et al., 2018; Vasconcelos et al., 2020); these patterns likely reflect greater geographic isolation afforded by montane habitats (e.g., Moritz et al., 2000; Smith et al., 2007), longer occupancy periods in climatically stable regions (Stephens & Wiens, 2003; Smith et al., 2007), or some combination of both. Plethodontid salamanders are no exception to this richness pattern, as salamander biodiversity peaks at mid elevations (Kozak, 2017), reflecting long occupancy periods for speciation (Kozak & Wiens, 2010). Yet, this phenomenon in species richness does not extend to patterns of phenotypic and ecological diversity in lungless salamanders (Adams et al., 2009). Instead, we find that diversification in lowland habitats is associated with a sharp acceleration in the rate of phenotypic evolution and more phenotypically dissimilar salamander assemblages, reflecting a proliferation of different microhabitat specialists. By contrast, microhabitat diversity wanes with elevation, with high-montane areas dominated by arboreal and terrestrial taxa. Therefore, mountains may simultaneously act as biological pumps for species richness and ecological brakes in terms of microhabitat specialist diversity and rates of phenotypic evolution, resulting in a decoupled response across different dimensions of diversity (Adams et al., 2009).

A question that emerges from our results is why phenotypic evolution accelerates at low elevation, given that species diversity peaks at higher elevation. Mountains affect the ecological diversity of lungless salamanders by imposing geological limitations and environmental constraints (Feder, 1983; Farallo et al., 2020). The geological properties of mountains render certain microhabitats less common at high elevations. For example, continuous swaths of karst formations (conducive for cave development) are prominent in the lowland plains of the Eastern and Central parts of the United States and in certain parts of Mexico (e.g., the Yucatan Peninsula) but are limited to small discontinuous tracts in montane regions, including the Appalachians and Northern Andes (Goldscheider et al., 2020). Likewise, aquatic habitat is less abundant at higher elevation (Dunne & Leopold, 1978; Leopold & Maddock, 1953; Montgomery & Dietrich, 1992). Mountain streams tend to be small and intermittent, further restricting the availability of aquatic habitat. Soil depth is likewise reduced along mountaintops (Heimsath et al., 1997), imposing spatial restrictions on fossorial habitat. Therefore, the ecological opportunity afforded by many microhabitats is reduced at high elevation and along mountaintops. Given that this pattern is an inherent geological property of mountains themselves, it may have far-reaching implications for ecological specialization and phenotypic evolution in montane organisms. Specifically, the ability for ecological opportunity to translate into evolutionary outcomes increases with geographic area (Frishkoff et al., 2022); in other words, the same geographic discontinuities that favor isolation and speciation also limit evolutionary access to certain habitat types and resources that could prompt phenotypic specialization.

Putting these pieces together, we find that high elevation assemblages are funneled toward terrestriality and arboreality (Figure 4), which are the two categories associated with the slowest rates of phenotypic evolution. We might expect salamanders to exploit alternative microhabitats in response to reduced terrestrial surface area at high elevation (Elsen & Tingley, 2015), but our results suggest this is not the case. Rather, several environmental and physiological factors also contribute to the reduced ecological diversity at high elevations. In the absence of lungs, plethodontid physiology requires a moist abiotic environment to facilitate cutaneous gas exchange and cool temperatures to keep metabolic rates low (Feder, 1983; Riddell et al., 2024). Temperatures are warmer at low elevation than at high elevation, and salamanders may seek buffered microhabitats like streams, caves, and rock crevices that provide cool conditions to maintain homeostasis (Feder, 1983). As elevation increases, temperatures cool, and the broad terrestrial landscape becomes more climatically amenable (Gade & Peterman, 2019). However, at even higher elevations, such as those in the Sierra Nevada and Sierra Madre (>3,000 m), temperatures continue to plunge, pushing salamanders closer to physiological limits. For example, solubility can restrict oxygen supply for aquatic organisms in cold environments (Verberk et al., 2011). As a result, salamanders may seek buffered refuge in terrestrial habitat, including vegetation in densely forested areas, where temperatures are consistently warmer than the ground (Klinges & Scheffers, 2021; Scheffers et al., 2013), instead of other microhabitats that offer cooler conditions (Ficetolo et al., 2018). Further, canopies appear to have more heterogeneous thermal regimes than the soil and ground-level, creating more overlap between lowland and upland areas; therefore, higher elevations may be thermally hospitable to arboreal species (Scheffers & Williams, 2018). As such, the asymmetrical distribution of microhabitat specialists across elevational gradients could also reflect shifting optimal thermoregulatory strategies (Kozak & Wiens, 2010). Geological and physiological processes likely operate together to constrain the availability and suitability, respectively, of many salamander niches at high elevation.

Although the Appalachian Mountains catalyzed much of the species-richness of lungless salamanders (Wilder & Dunn, 1920; but see Ruben & Boucot, 1989; Beachy & Bruce, 1992; Ruben et al., 1993), we show that much of their phenotypic and ecological diversity arose following their expansion into lowlands. Several major lineages expanded from highlands into surrounding interior and coastal lowlands during the early Paleogene (*e.g.*, *Eurycea* and *Gyrinophilus*; Wray & Steppan, 2017). Lowland expansion was made possible by receding sea levels and subsequent exposure of the lowland landscape (Miller et al., 2005; Wray & Steppan, 2017). This shift to lower elevation provided access to a wider range of available microhabitats, which prompted rapid phenotypic evolution and expansion of ecological diversity (Figures 2–4). A concomitant bout of widespread origination of secondary functional innovations likely further enabled exploration of these microhabitats (Figure 1; Table 1). Key secondary functional innovations associated with transitions in elevation include ballistic tongues (Deban et al., 2020), miniaturization (Rovito et al., 2013), limb reduction (Parra-Olea & Wake, 2001), and the skeletomuscular suite in *Desmognathus* (Schwenk & Wake, 1993). Yet, there were also some idiosyncrasies in how functional innovation shaped evolution. For example, expanded

interdigital webbing is associated with transitions to cave (*Hydromantes*) and arboreal environments (*Chiropterotriton* and *Bolitoglossa*), but only the former also exhibits an accompanying shift to elevated rates of phenotypic evolution (Table 1). Further, within *Chiropterotriton*, the cave-adapted *C. magnipes* exhibits elevated rates of phenotypic evolution (Figure 1). Taken together, these patterns suggest that expanded interdigital webbing may have more dramatic effects on phenotypic evolution if they are also associated with cave rather than arboreal habitats. This context-dependent effect of innovation on evolution may be more widespread; in lizards, for example, some sources of ecological opportunity only prompted an acceleration in evolution when they arose in combination with other sources of opportunity (Alencar et al., 2024). Therefore, the macroevolutionary signatures of some functional innovations of salamanders (and other organisms) may ultimately hinge upon the ecological setting in which they arise.

An ancient adaptive radiation of salamanders

Lungless salamanders have been widely recognized as an innovation-rich lineage, and that those innovations were important to local bouts of adaptation to different niches and environmental conditions (Bruce, 1996; Deban et al., 2020; Parra-Olea & Wake, 2001; Rovito et al., 2013; Schwenk & Wake, 1993; Wake, 1987, 2009). Our results corroborate these ideas, showing that innovations and transitions into lowlands largely operated together and imparted classic macroevolutionary signatures of adaptive radiation (Glor, 2010; Schluter, 2000; Simpson, 1953; Yoder et al., 2010). Lungless salamanders diversified in the immediate aftermath of the K-Pg boundary, possibly following a mass extinction across Caudata (Jetz & Pyron, 2018; Vieites et al., 2007). A conspicuous early proliferation of functional innovations likely set the stage for salamanders to diversify along elevational gradients into different microhabitats (Figure 4). Several functional innovations arose coincident with Plethodontidae, such as direct development that facilitated the exploration of terrestrial habitat by releasing salamander reproduction from the aquatic realm (Wake & Hanken, 1996) and nasolabial grooves that facilitated olfaction while dynamically switching between aquatic and terrestrial conditions (Bonett & Blair, 2017; Brown & Martof, 1966). Thus, early plethodontid salamanders may have been well-equipped to respond to subsequent ecological opportunity.

Heterogeneity in evolutionary rate, either temporally or among lineages, is a key macroevolutionary signature of ecological opportunity (Glor, 2010; Simpson, 1953; Schluter, 2000). Rates of phenotypic evolution varied an order of magnitude in response to elevation alone (Figures 1 and 2) and 68.8-fold overall (Figure S8). For comparison, using a similar phenotypic dataset and the same phylogenetic comparative method, Burress & Muñoz (2022) found that rates of phenotypic evolution varied only about 3.3-fold across *Anolis* lizards, a classic case of adaptive radiation associated with geographic transitions (e.g., between the mainland and islands) and functional innovations (e.g., adhesive toepads). In fact, several plethodontid genera exhibit more rate heterogeneity than *Anolis* (i.e., *Eurycea*, *Plethodon*, *Bolitoglossa*, and *Thorius*). In cichlids, rates of jaw evolution vary up to 6.6-fold in Lake Malawi (Hulsey et al., 2010), up to

47.3-fold in Lake Tanganyika (Ronco & Salzburger, 2021), and up to 12.7-fold in the Americas (Burress et al., 2020). The magnitude of the "elevation effect" on salamander phenotypic evolution is on par with or far greater than the "coral reef effect" on marine fishes (up to 8.5-fold; Price et al., 2011; 2013), the "lake effect" on cichlid fishes (up to 10-fold; Burress & Muñoz, 2023; Hulsey et al., 2010), the "island effect" on anole lizards (often negligible but up to 6-fold; Burress & Muñoz, 2022; Pinto et al., 2008; Poe et al., 2018; Salazar et al., 2019), and the latitude effect on marine fishes (up to 2-fold; Burns et al., 2024). Therefore, phenotypic rate heterogeneity and the "mountain effect" in plethodontid salamanders are on par with or exceed the effect of major biogeographic shifts in various adaptive radiations.

Caveats

The complex evolutionary history of lungless salamanders, ranging from putative adaptive radiations (e.g., *Desmognathus*, *Thorius*, etc.; Rovito et al., 2013; Weaver et al., 2020) to classic non-adaptive radiations (e.g., *Plethodon*; Kozak et al., 2006) and seemingly conflicting patterns in which both montane and lowland environments can drive their diversification (Parra-Olea & Wake, 2001; Wake, 1987) motivated this study. Although we accounted for unknown sources of variation where feasible (in the phenotypic rate and transition models), many innovations are likely unknown, or their phylogenetic distribution is poorly understood. For example, most of the functional innovations posed in the literature are synapomorphies of named clades (Schwenk & Wake, 1993) or otherwise characterize most members of named clades (Kozak et al., 2005; Rovito et al., 2013), leaving room for bias at other phylogenetic scales. Although some degree of evolutionary lag following exposure to ecological opportunity may be expected (Burress & Tan, 2017; Burress & Muñoz, 2023), delayed rate shifts may be prone to false positives such that they truly occur in response to unknown factors. Reconciling the relative contributions of innovations and elevation to the diversification of lungless salamanders was a major goal of our study, yet our results suggest further factors driving key variation. For example, few innovations spurred rate shifts across all the descending lineages (e.g., IDD and KT within *Desmognathus*); more commonly, these macroevolutionary signatures manifested in 50%–75% of the descending lineages, and in two instances, less than 50% (Table 1). This variability may suggest context-dependence of the innovations, interactions with other factors, misidentification of the source of the rate variation, or arise due to non-random incomplete taxon sampling.

Conclusions

Lungless salamanders are characterized by intriguing evolutionary contrasts. Whereas some lineages rapidly diversified, others generated biodiversity more slowly (Bruce, 1996; Kozak et al., 2006; Martin & Richards, 2019; Rundell & Price, 2009; Rovito et al., 2013; Wake, 1987; Weaver et al., 2020). Plethodntid lineages with high rates of species generation need not have fast rates of evolution, and lineages that rapidly accumulate morphological diversity often have surprisingly slow speciation (Adams et al., 2009). These con-

trasts can be contextualized by isolating and quantifying the effects of geographic transitions and functional innovations on salamander diversity. Montane environments certainly function as engines for lungless salamander biodiversity (Beachy & Bruce, 1992; García-Rodríguez et al., 2021; Wake, 1987, 2009; Wilder & Dunn, 1920), but much of the phenotypic and ecological diversity arose following expansion into lowland areas (Figures 1, 2, and 4), as sub-lineages adapted to different ecological niches (roughly corresponding to nominal genera; Figure 5). Receding sea levels exposed lowland areas during the early Paleogene (Miller et al., 2005; Wray & Steppan, 2017), providing access to an expanded range of microhabitats for montane salamanders. Numerous lineages of salamanders invaded these areas, often associated with functional innovations (Figure 1). These patterns are consistent with other ecologically diverse continental radiations that diversified along prominent environmental gradients (Burress & Hart, 2024; Lopez-Fernandez et al., 2013). By considering the natural multiplicity of sources of ecological opportunity, our study improves our understanding of how environmental gradients and innovations interacted to shape the remarkable diversity of lungless salamanders.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Data for this study have been archived in the Dryad data repository (https://doi.org/10.5061/dryad.mpg4f4rdw). Scripts are archived on Zenodo (https://doi.org/10.5281/zenodo.17905776).

Author contributions

E.D.B. and M.M.M. designed the study. E.D.B. and M.R.G. collected data. E.D.B. analyzed data. E.D.B. and M.R.G. wrote the manuscript. All authors contributed substantially to the preparation of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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