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Physiology is a hidden dimension of diversity in the radiation of woodland salamanders

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Morphological evolution can be explosive, producing visually spectacular adaptive radiations like Caribbean anoles, Malagasy vangas, and African Rift Lake cichlids. Yet morphological stasis, the long-term retention of a conserved body plan, is often observed across evolutionary radiations. Woodland salamanders (*Plethodon*) are a classic example of such "nonadaptive" radiation, characterized by prolific speciation alongside morphological stasis (i.e., limited morphological divergence), often attributed to phylogenetic conservatism in their climatic and microhabitat niches. However, the multidimensional nature of phenotypes and the niche means that adaptive evolution in less apparent traits can occur even when morphology appears static. We investigated whether woodland salamanders exhibit adaptive divergence in a less conspicuous phenotypic axis—specifically, physiology—and compared patterns and rates of trait evolution to those of morphological traits. We found that most physiological traits are associated with climatic variation and exhibit elevated rates of evolution, high trait disparity, and more frequent shifts in adaptive optima than morphological traits. In particular, skin resistance to water loss, metabolic rate, and cold tolerance exhibit evolutionary signatures of adaptive radiation. Notably, morphology is not entirely static: Some traits show climatic associations, several exhibit localized shifts, and evolutionary rates exceed those of slower evolving physiological traits, such as heat tolerance. Biological systems, as evidenced by woodland salamanders, are not exclusively "conserved" or "labile" in their evolution, and this system illustrates how the same features that limit morphological divergence may also facilitate physiological evolution. Woodland salamanders exemplify how adaptive radiation can proceed despite outward similarity.

evolutionary stasis | niche conservatism | adaptive radiation | Plethodontidae | macroevolution

Morphological evolution is conspicuously lopsided across the tree of life (1). Some lineages undergo visually dramatic adaptive radiations (e.g., African rift lake cichlids, Hawaiian silverswords, and Caribbean anoles) (2), whereas others are species-rich but strikingly uniform, characterized by morphological stasis over million-year timescales. In several lineages of plethodontid (lungless) salamanders, for example, extensive speciation has occurred with little accompanying divergence in body shape among species (3), a phenomenon often termed "nonadaptive" radiation (2, 4–6). These contrasting outcomes are typically attributed to different mechanisms underpinning speciation, specifically divergent selection in adaptive radiations and geographic isolation in "nonadaptive" radiations (2, 6, 7). Although phenotypic diversification is inherently multidimensional (involving divergence in morphology, physiology, behavior, life history, or their combinations), most studies focus on patterns of morphological evolution (2). Early comparative work suggested that physiological traits may diverge even when morphology and ecological structure remain relatively conserved (8), hinting at the idea that evolutionary lability can differ among trait dimensions.

From this perspective, some "nonadaptive" radiations may exhibit adaptive diversification in nonmorphological traits because lineages can remain conserved along one niche axis while diverging along another. For example, evolutionary lability in structural microhabitat use prompted morphological diversification across Caribbean *Anolis* (i.e., "ecomorph" evolution) (9). Yet, species within the same ecomorph share structural habitat use and morphology, but often diverge into different microclimates, with associated diversification in thermal physiology (10). A single lineage may therefore span the continuum between adaptive and nonadaptive radiation, as well as niche lability and niche conservatism, blurring the boundary between these phenomena (2). Expanding our perspective by incorporating multiple trait types could reveal unexplored dimensions of adaptive evolution, refining our understanding of how biodiversity arises, challenging assumptions

Significance

The Appalachian Mountains harbor a global hotspot of salamander diversity. Within this hotspot, woodland salamanders (*Plethodon*) are species-rich, yet morphologically conserved, making them a classic example of "nonadaptive" radiation. We investigate whether *Plethodon* exhibits adaptive diversity in another dimension—physiology. By integrating measurements of skin resistance to water loss, metabolic rate, and thermal physiology for 30 *Plethodon* species, we show that most physiological traits are highly labile and diversified at rates that outpace morphology. Physiological divergence, therefore, represents an underappreciated axis of adaptive diversity in *Plethodon*. More broadly, radiations can span a continuum in which adaptive and nonadaptive dynamics coexist, a complexity best revealed through a multidimensional trait perspective.

The authors declare no competing interest.

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about the rigidity of the diversity continuum, and reframing our interpretations of evolutionary stasis and lability.

Salamanders are often noted for long-term conservatism in body form, with a broadly similar body plan persisting for >100 million years across most lineages, apart from obligate paedomorphs (3, 11–14). Yet, morphological stasis may belie phenotypic lability in other traits. Many radiations show mosaic evolution, in which some traits diversify rapidly while others remain relatively conserved (15–17). In African cichlid fishes, for example, oral jaw traits show rapid adaptive divergence (18), whereas the modified pharyngeal jaw exhibits reduced evolutionary independence and slower rates of evolution (19). Morphological similarity can therefore mask ecologically relevant divergence in other traits (20). The challenge resides in empirically quantifying diversity across trait types and integrating them into a cohesive evolutionary framework. Few studies have compared evolutionary patterns across trait classes (21) and such integrative datasets remain uncommon at macroevolutionary scales, especially for physiology, because comparable measurements across many species are experimentally intensive (22, 23).

In this study, we assess whether physiology represents a "hidden" dimension of adaptive radiation in woodland salamanders (Genus *Plethodon*), a clade (58 species) of lungless amphibians long considered a classic case of "nonadaptive" radiation, with evidence of early lineage accumulation despite limited morphological disparity (5, 6). Most diversity is concentrated in the southern Appalachian Mountains of eastern North America, a global hotspot of salamander diversity (24). Despite rapid speciation, woodland salamanders are widely characterized as morphologically conserved (11, 25); *Plethodon* exhibits low morphological disparity, quantified as variation among species in overall body form using multiple body measurements, compared with other plethodontid clades (*Plethodon*: ~0.10 to 0.21; *Eurycea* ~0.94; *Desmognathus*+*Phaeognathus* ~1.05) (26). Retention of shared ancestral climatic niches, reflecting in part exclusive reliance on cutaneous respiration, restricts woodland salamanders to cool, moist montane environments, which promoted speciation by vicariant isolation across discontinuous high-elevation habitats in the Appalachians (27). Despite having conserved macroclimatic niches, woodland salamander species differ in microclimate use (28), which could allow for fine-scale physiological differentiation. Additional features may also favor physiological lability: Woodland salamanders exhibit direct development, a life-history transition that increased exposure to variable thermal and hydric terrestrial conditions compared to their ancestral aquatic habitats (29, 30). Indeed, *Plethodon* vary in skin resistance to water loss (the skin's physiological resistance to evaporative water loss), metabolic rate, and thermal physiology; these traits are associated with microclimatic variation, elevational gradients, and subtle morphological differences (31–34). Morphological conservatism likewise merits deeper exploration, as prior analyses were based on external linear measurements (25, 35, 36); however, CT skeletal scans may reveal phenotypic variation not externally evident (37–39).

Here, we employed a multidimensional approach to investigate the potential signatures of adaptive radiation in woodland salamanders and how those signatures vary among trait dimensions. We quantified and compared physiological and morphological diversity from 30 *Plethodon* species from eastern North America. We assembled a dataset of species-level variation in skin resistance to water loss, metabolic rate, thermal physiology (heat tolerance, CT_{max}; cold tolerance, CT_{min}; and selected temperature, T_{sel}), and we tested whether seasonal plasticity and laboratory acclimation influenced physiological results. Using micro-CT scanning, we also gathered data on morphology (body size, relative limb and trunk size, and skull shape). We first investigated patterns of phenotypic specialization by quantifying the evolutionary relationships between climatic conditions and morphological and physiological traits. We then used phylogenetic comparative methods to assess how patterns and rates of evolution vary among traits. Specifically, we tested whether physiological traits exhibit more punctuated bursts of evolution than morphological traits, how adaptive differentiation varies among traits (testing whether the number of optima is higher for physiological than morphological traits), and how rates of evolution vary among traits (testing whether physiological evolution outpaces morphological evolution). Our results reveal that specialization of most physiological traits is common and highly labile across the woodland salamander radiation. We also found striking differences in the tempo and mode of physiological and morphological evolution and that physiological traits usually exhibit more macroevolutionary signatures of adaptive radiation. These findings upend the prevailing view that woodland salamanders represent a nonadaptive radiation (5, 40, 41) and instead offer a more nuanced interpretation that recognizes the complementary roles of vicariance and adaptive specialization. Multidimensional trait perspectives afford a broader vantage point on the processes that generate and limit the evolution of biodiversity (2).

Results and Discussion

Woodland Salamanders Exhibit Both Physiological Specialization and Macroclimatic Niche Conservatism. *Plethodon* species exhibit fine-scale physiological divergence aligned with both micro- and macroclimatic niches (Fig. 1 and *SI Appendix*, Tables S1–S3 and Figs. S1 and S2) (28). Species from warmer macroclimates exhibited higher skin resistance to water loss (Fig. 1*B*; slope = 0.186 ± 0.082 SE, $P = 0.025$), which can buffer them from dehydrating in relatively dry air (32, 33, 42). Skin resistance also weakly decreased with annual temperature range in the best-supported model (BIO7 slope = −0.182 ± 0.139 SE, $P = 0.190$; *SI Appendix*, Table S1). These patterns were broadly consistent in complementary analyses using the full individual-level data that accounted for phylogeny and measurement error, indicating that the main species-level inferences were robust to within-species variance and sampling error, although support for specific predictors varied (*SI Appendix*, Tables S11–S13). Trait associations with climate are presented trait-by-trait, and evolutionary correlations among traits were generally weak (median $|r| \approx 0.20$; *SI Appendix*, Table S14), although a few trait pairs showed moderate correlations. It is important to note that such physiological specialization is insufficient to overcome the barriers to dispersal presented by intervening valleys that are typically warmer and drier than adjacent uplands (27, 43). Lungless salamanders still have relatively low skin resistance and high hydric costs of respiration compared to other tetrapod taxa (44, 45); the observed patterns of physiological specialization do not contradict the general pattern of macroclimatic niche conservatism long appreciated in *Plethodon* (27, 46). Likewise, other behavioral and ecological mechanisms such as territoriality and priority effects (where incumbents have a competitive advantage that reduces the success of newcomers) may also limit the ability of individuals to disperse into new habitats (47, 48). In other words, fine-scale physiological diversity arose without redefining the broad-scale environmental and competitive boundaries that isolate species.

Metabolic rate exhibited positive relationships with climate, with higher rates in species from warmer environments (BIO1; slope = 0.063 ± 0.010 SE, $P < 0.001$) and from environments with warmer wet-season temperatures (BIO8; slope = 0.012 ±

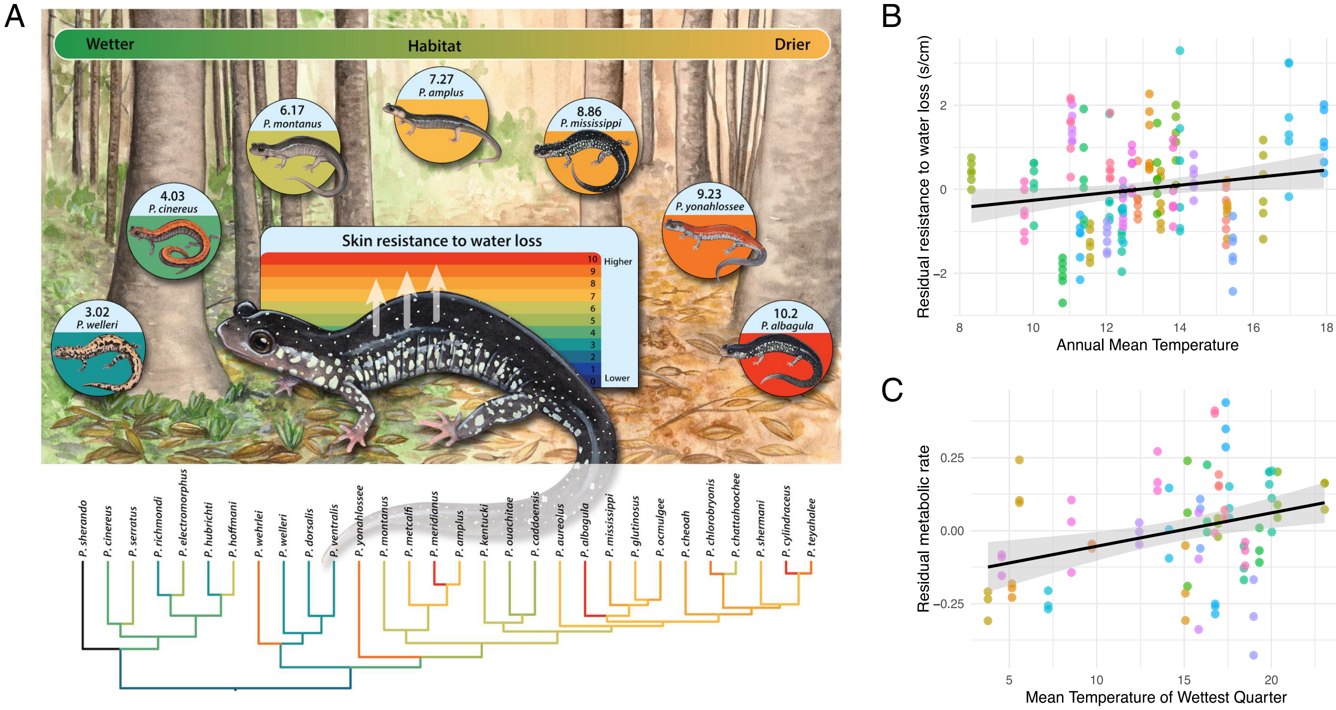

Fig. 1. Physiological diversity in woodland salamanders and its relationship to environmental conditions. (*A*) Illustration of skin resistance to water loss (r_i, s/cm) across *Plethodon* species, corresponding to a habitat gradient ranging from wetter to drier environments. The branch color on the phylogeny denotes the mean r_i values (s/cm) for each species. (*B*) Adjusted relationship between r_i (s/cm) and annual mean air temperature (°C) for each species, based on WorldClim data. Each point is an individual salamander, with r_i adjusted for mass, experimental temperature, and vapor pressure deficit. Each color is a different species. The black line visualizes the adjusted relationship from the model; gray shading is the 95% CI (*SI Appendix*, Table S1). (*C*) Adjusted relationship between metabolic rate and mean temperature of the wettest quarter, representing a second climatic dimension associated with physiology. Each point represents an individual salamander, with metabolic rate adjusted for mass and experimental temperature. Colors denote different species. The black line visualizes the fitted adjusted relationship from the model; gray shading indicates the 95% CI (*SI Appendix*, Table S2). Illustration credit: Life Science Studios.

0.005 SE, P = 0.02; Fig. 1*C* and *SI Appendix*, Fig. S3 and Table S2). Within this broad pattern, species in the *P. glutinosus* group ("slimy" salamanders) generally exhibited lower metabolic rates across experimental temperatures (*SI Appendix*, Fig. S2*A*), although this pattern weakened at the warmest temperature. Thermal sensitivity (Q_{10}) of metabolic rate also varied across lineages, with slimy salamanders exhibiting relatively high thermal sensitivity (*SI Appendix*, Fig. S2 *B* and *C*). This clade is associated with relatively warm (and often dry) habitats; elevated Q_{10} values suggest lineage-specific metabolic tuning to thermal environments, potentially reflecting adaptation to higher metabolic demands in warmer habitats (49, 50).

We also found that cold tolerance (CT_{min}) in *Plethodon* showed evidence of decreasing with environmental temperature (BIO8; slope = −0.026 ± 0.013 SE, P = 0.048; *SI Appendix*, Table S3) and some evidence of increasing with higher evaporative demand of the microclimatic air, measured as vapor pressure deficit (VPD; slope = 1.130 ± 0.505 SE, P = 0.034; *SI Appendix*, Table S3), consistent with broad patterns across ectotherms and reflecting the need to maintain locomotor function in cooler habitats (51–53). T_{sel} was linked to climate at both broad and local scales, increasing in habitats with warmer wet-season temperatures (BIO8; slope = 0.062 ± 0.023 SE, P = 0.0104; *SI Appendix*, Table S3) and drier microclimates (VPD; slope = 1.689 ± 0.907 SE, P = 0.0739; *SI Appendix*, Table S3). Salamanders occupy a wide temperature range in thermal-gradient assays, rather than converge on a single, narrow optimum. The body temperatures selected in a thermal gradient can also vary with the fine-scale thermal heterogeneity of the gradient itself, underscoring the importance of considering gradient structure when interpreting T_{sel} (54). Heat tolerance exhibited no clear associations with either micro- or macroclimate as models with climatic predictors were not better supported than the null (*SI Appendix*, Table S3). This pattern is consistent with two nonmutually exclusive explanations. First, upper thermal limits are often thought to be evolutionarily constrained by biochemical and physiological limits, which can restrict divergence in heat tolerance across related species (51, 55). Second, *Plethodon* are nocturnal and retreat to cool underground refugia during the hottest times of day, likely reducing exposure to extreme surface temperatures and limiting selection on heat tolerance (56, 57).

Of the linear morphological traits examined (body size, forelimb length, hindlimb length), only body size (SVL) was clearly associated with climate, showing a positive association with mean diurnal range (BIO2; slope = 6.456 ± 2.601 SE, P = 0.020; *SI Appendix*, Table S4) and local air temperature (AIR.TEMP; slope = 1.097 ± 0.258 SE, P < 0.001; *SI Appendix*, Table S4). This indicates that *Plethodon* are larger in warmer environments, contrary to the expectation under Bergmann's rule (58). Inverse or inconsistent clines are common in amphibians (and other vertebrate ectotherms) (59–61), likely because body size is shaped by many factors beyond heat conservation, such as activity patterns, moisture availability, and primary productivity. For example, warmer environments often allow for longer activity periods, which may favor larger body sizes (62, 63). In contrast to body size, climatic variables exhibited no clear associations with size-corrected residual limb length (*SI Appendix*, Table S4). This pattern is not unexpected given that relative limb proportions are strongly shaped by functional demands associated with microhabitat use rather than climate (64–66). In anole lizards, for example, species with comparable limb proportions use similar structural microhabitats, but diverge in thermal niche space (10, 21, 67). At the same time, anole species specialized to the same microhabitat are often smaller in colder environments (68), as observed here.

Physiology Is a Key Dimension of Adaptive Diversity in Woodland Salamanders. Adaptive radiation can be associated with several macroevolutionary signals in trait evolution, including i) pronounced "bursts" of trait disparity, ii) accelerated rates of trait evolution, and iii) heterogeneity in selective regimes, which may manifest as multiple phenotypic optima (69, 70). We found that physiological traits consistently exhibited more complex macroevolutionary patterns, characterized by more adaptive optima, stronger convergence, and faster rates of evolution than morphological traits.

Our disparity-through-time (DTT) analyses show that most traits conform to Brownian motion (BM) expectations, suggesting gradual, unconstrained divergence (Fig. 2). Thermal physiology deviates from the BM envelope in more recent time slices, both in the multivariate (Fig. 2*D*) and univariate analyses (*SI Appendix*, Fig. S4). Consistent with this pattern, branch-specific rates for thermal physiology show heterogeneity (Fig. 2*D*), including lower rates along the *P. caddoensis*–*P. ouachitae* lineage and higher rates on the *P. aureolus* branch. This within-clade disparification indicates either convergence (e.g., repeated transitions into cool habitats) or rapid divergence in multiple lineages. Both scenarios are plausible, as convergence can lower phylogenetic signal (*SI Appendix*, Table S5), and rate disparity is largely restricted to a few recently diverged species in the *P. glutinosus* ("slimy" salamander) lineage (Fig. 2*D* and *SI Appendix*, Fig. S5).

Physiological traits show patterns consistent with a more rugged adaptive landscape, as indicated by a greater number of inferred shifts to distinct phenotypic optima in hydric, thermal, and metabolic traits (seven, five, and six transitions, respectively) when compared to morphological traits (four shifts) (Fig. 3 *A*–*D*). Shifts represent evolutionary transitions away from an ancestral or background trait regime into distinct adaptive optima, suggesting divergence in selective pressures or ecological conditions. In some cases, morphological and physiological shifts align: An early increase in skin resistance to water loss coincides with an increase in body size near the origin of the *glutinosus* group, suggesting coordinated divergence within this species-rich lineage. This coupling is consistent with selection on water balance, as higher skin resistance and a lower surface area-to-volume ratio both reduce moisture loss. The inferred placement of these shifts near the origin of the *glutinosus* group is broadly consistent with late Miocene–early Pliocene environmental change in eastern North America, when prolonged dry periods may have limited forests to higher elevations, increased isolation among eastern *Plethodon* lineages, and prompted adaptive divergence in water balance (25, 71). Phylogenetic signal is low for thermal physiology and metabolic rates (*SI Appendix*, Table S5; $K \approx 0.21$ to 0.29); correspondingly, different optima for these traits are occupied by one or a few species, indicating high phenotypic divergence among close relatives (Fig. 3). Phylogenetic signal for skin resistance to water loss

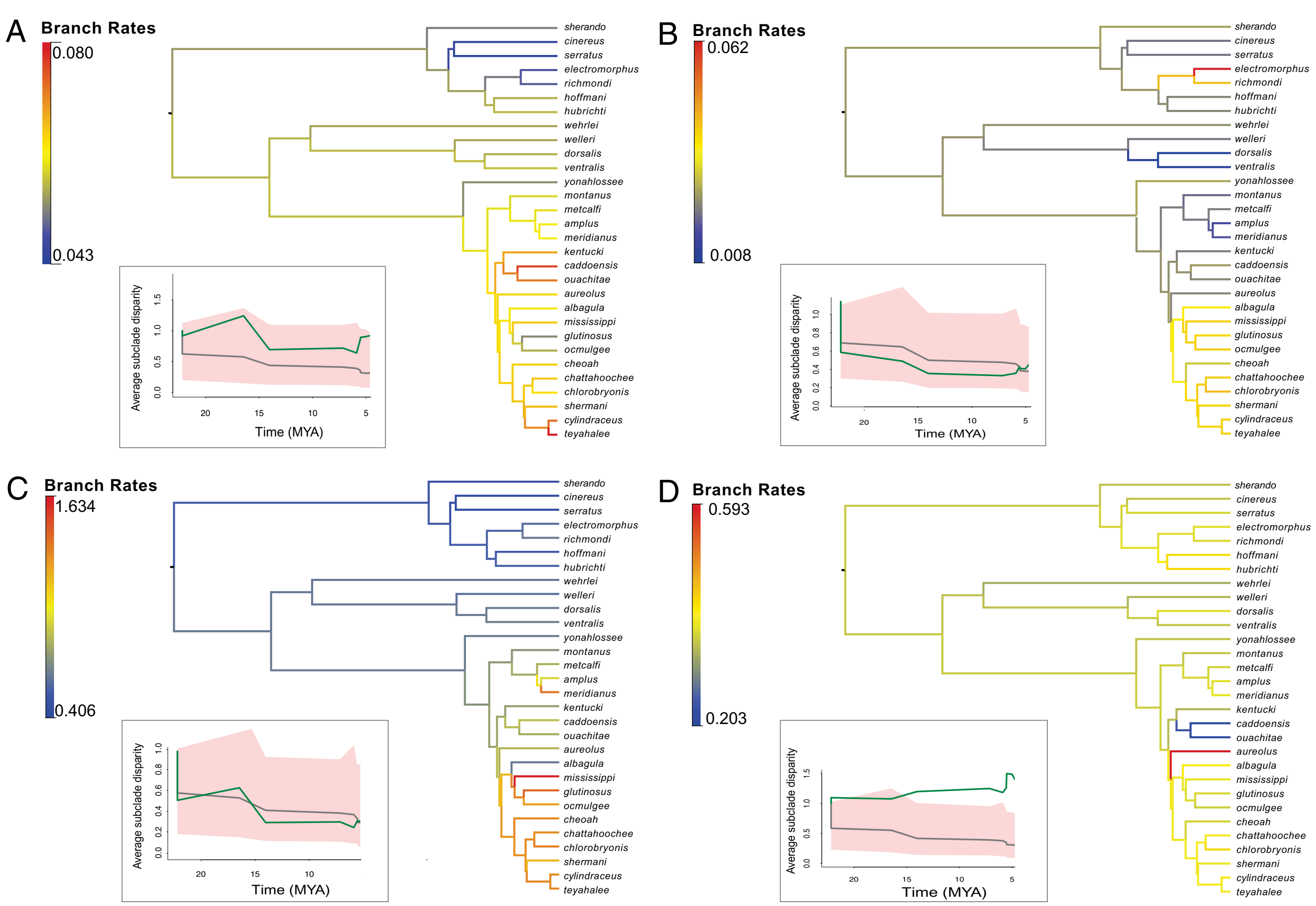

Fig. 2. Timing and Rates of Physiological and Morphological Evolution in Woodland Salamanders. Background rates of evolution for (*A*) metabolic rate, (*B*) linear morphology, (*C*) skin resistance, and (*D*) thermal physiology are mapped onto the *Plethodon* phylogeny [color ramp denotes relative rates, ranging from slower (cooler colors) to faster (warmer colors)]. Insets show DTT plots, illustrating the accumulation of trait variation relative to Brownian motion (BM) expectations. In each DTT plot the gray line shows the median expectation, the green line indicates the observed disparity, and the pink shading indicates the 95% CI of BM simulations. Values outside the shaded region indicate departures from Brownian expectations.

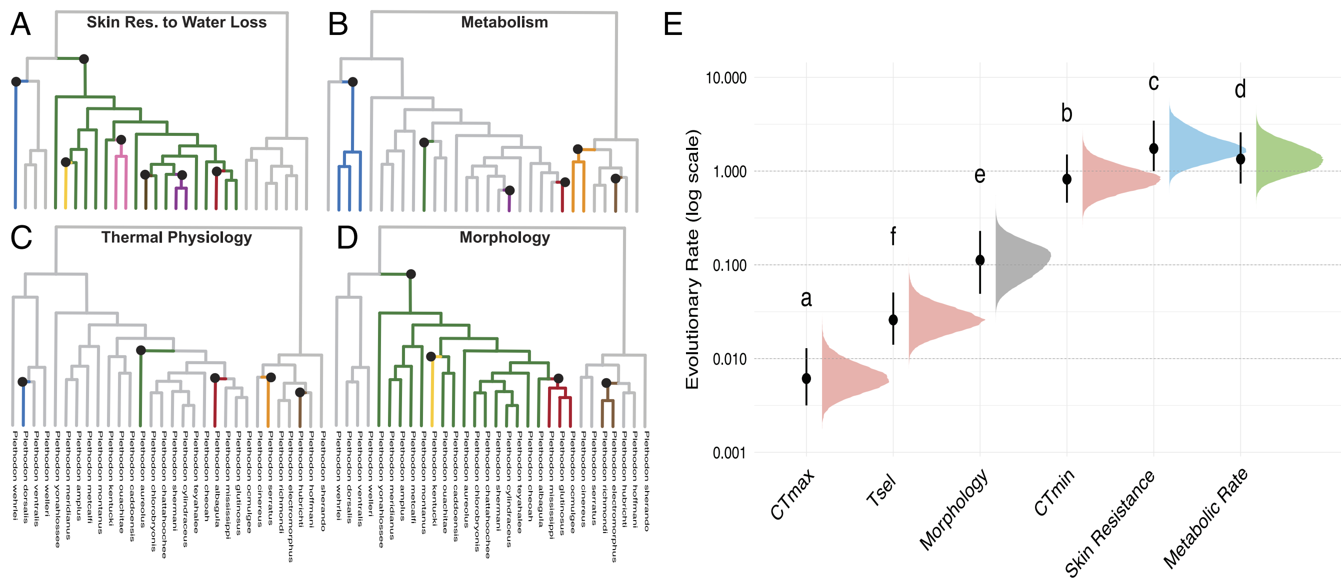

Fig. 3. More Adaptive Peak Shifts and Higher Rates of Evolution in Physiological Traits. Adaptive peak shifts inferred using the l1ou model across four trait categories: (*A*) Skin resistance to water loss (r_i), (*B*) metabolic physiology, (*C*) thermal physiology, (*D*) linear morphology. Black circles indicate nodes inferred to represent transitions to alternative adaptive optima. (*E*) Distributions of evolutionary rates (σ^2) across trait categories, shown as density plots with median values (points) and 95% intervals (bars). Skin resistance to water loss, metabolic, and CT_{min} traits evolve faster than morphological traits, CT_{max}, and T_{sel}. All groups differ significantly ($P < 0.05$, Dunn's test with Bonferroni correction). Colored branches in *A*–*D* denote distinct inferred optima (colors are arbitrary). In *E*, colors denote trait category: thermal = red, morphology = gray, hydric = blue, metabolic = green.

($K \approx 0.35$ to 0.54) was generally higher than for metabolic rate or thermal traits (*SI Appendix*, Table S5). This pattern is consistent with prior work reporting strong phylogenetic signal in amphibian cutaneous resistance to evaporation (72) and may reflect greater conservation of skin-barrier traits that influence water loss, including stratum corneum structure and superficial lipid or secretory layers (44). As with skin resistance, phylogenetic signal for morphological traits was also often moderate to high ($K \approx 0.4$ to 0.7), but varied among traits (*SI Appendix*, Table S5), with proximal elements generally showing stronger signal than distal elements. Similar heterogeneity has been observed in salamander limb bones more broadly; different components of the limb can evolve in a decoupled manner under varying functional demands (73).

Estimated rates of evolution were often higher for physiological than morphological traits: skin resistance to water loss, metabolic rate, and cold tolerance evolved most rapidly, while morphological traits fell in an intermediate range relative to the full suite of traits measured, both when summarized as multivariate data (Fig. 3*E*) and in trait-by-trait comparisons (*SI Appendix*, Fig. S6). These differences were not explained by within-species variance/measurement-error proxies derived from the individual-level data, suggesting that the faster evolution of physiological traits is unlikely to be an artifact of greater measurement error or within-species scatter (*SI Appendix*, Table S15). While comparing rate differences among trait types is complicated by different units, they cannot be explained by differences in scale, as Kvålseth's coefficient of variability (KCV) does not mirror the large differences in inferred evolutionary rates (*SI Appendix*, Fig. S7). Heat tolerance (CT_{max}) and the selected temperature (T_{sel}) were notable for their exceptionally slow rates of evolution. As described above, relatively slow evolution is expected for traits on the upper end of the thermal performance curve, where performance declines steeply and biochemical constraints are strong (51, 55, 74). In *Plethodon*, this pattern is likely reinforced by limited exposure to high temperatures, as nocturnality and behavioral buffering reduce selection on heat tolerance (75, 76). In contrast, cold tolerance is often labile in ectotherms and closely tracks minimum environmental temperatures (17, 68, 77). This physiological fine-tuning of cold tolerance is likely driven by the nocturnality of woodland salamanders: Such lability can help ensure effective locomotion and predator avoidance when engaging in fitness-based activities.

Hydric physiology stands out as evolving rapidly along a topographically complex landscape. Of the seven adaptive shifts observed, six are concentrated within the slimy sublineage (Fig. 3*A*), which also exhibits the fastest rates of speciation in *Plethodon* (5, 78) and contributes substantially to the southern Appalachian Mountains' salamander biodiversity. This pattern is consistent with diversification across broader, warmer macroclimates, with hydric shifts potentially facilitating that expansion. Where ranges overlap, physiological specialization may lessen competition by allowing species to partition microclimatic space, packing diversity into a relatively small geographic footprint. Consistent with this possibility, competition is known to restrict habitat use and reduce activity time in plethodontids. For example, *Plethodon cinereus* has been observed excluding other species from preferred microhabitats (79–81) and may restrict endemic species like *P. hubrichti* and *P. sherando* to suboptimal habitats (82). Similarly, another North American plethodontid, *Desmognathus monticola,* reduces its activity in the presence of competing *D. fuscus* (83). In this context, physiological divergence may help reduce niche overlap and promote coexistence by limiting competition among species.

Prior studies have long emphasized the morphological conservatism of woodland salamanders (11, 25–27). Recent comparative analyses of salamander locomotor traits likewise find relatively constrained limb diversification in terrestrial lineages, including *Plethodon* (84). Our analyses generally align with this pattern, but evolutionary rates nonetheless varied across skeletal elements (Fig. 3*E*), with some hindlimb traits, particularly the femur, evolving faster than several forelimb traits. Hindlimb length is strongly associated with structural habitat use (85, 86). Although *Plethodon* species are generally highly terrestrial, some species vary in the degree to which they use other microhabitats, particularly rocky substrates (87); the relatively faster rates of femur evolution may reflect shifts in habitat use among species, although this remains to be explored. We also detected several optima shifts in trunk and limb proportions that may indicate adaptive divergence in morphology, particularly in the slimy salamanders (Fig. 3*D*). Likewise, there were several bouts of localized adaptive divergence of cranial morphology into alternative optima (*SI Appendix*, Fig. S8). Cranial divergence among woodland salamander species has been previously documented (88); when *Plethodon cinereus* and *P. hoffmani* (both in the *cinereus* clade) co-occur, jaw shape adjusts for greater force in the former species and for greater speed in the latter (89). Notably, these two species occupy the same physiological optima and belong to a clade that exhibits slower rates of physiological evolution than the "slimy" clade. Instead of microclimatic differentiation, these species may reduce overlap through behavioral differences in microhabitat use or in activity patterns, such as differential surface activity in relation to moisture availability (57, 90). *Plethodon* salamanders share a relatively conserved body plan; nevertheless, we find that fine-scale morphological evolution may have meaningfully contributed to the lineage's diversity, a pattern that likely becomes obscured when compared to more morphologically diverse salamander radiations, such as those with paedomorphic representatives (13, 91).

Greater observed lability in physiological traits may reflect plastic responses to environmental variation (92–94). However, this seems unlikely in our case, as neither day of collection in the field (reflecting potential seasonal effects) nor time in the lab (reflecting acclimation time) significantly influenced our physiological estimates (*SI Appendix*, Figs. S9-S10). These analyses suggest that species-level differences driven by evolutionary change likely outweigh the influence of plasticity across species, seasons, and geography. Moreover, certain morphological traits can also exhibit plasticity in response to developmental temperature, a topic that has received comparatively less attention (95). Adaptive plasticity, including developmental effects, is an additional dimension of physiological diversity in woodland salamanders warranting future work.

The Value of Integrating Multidimensional Perspectives Into Diversification Studies. We do not suggest that all nonadaptive radiations are adaptive radiations awaiting discovery, or that adaptive radiations necessarily harbor cryptic signals of nonadaptive divergence. Many adaptive radiations, for example, are multidimensionally diverse, characterized by iterative bouts of phenotypic specialization (69, 96). Our results instead highlight an important asymmetry. Whereas detection of ecologically relevant trait evolution can be considered sufficient to infer adaptive radiation, finding no such divergence in a limited trait set does not demonstrate that radiation is wholly "nonadaptive"; only a comprehensive, multitrait assessment can do that (2, 69). Inferences about diversification can depend on the trait lens applied because conservatism and lability may unfold simultaneously within a radiation. Rather than placing lineages along a single continuum of diversity, our approach shows that different traits tell fundamentally different evolutionary stories within the same radiation.

Categorizations like "adaptive" and "nonadaptive" radiation, as well as "niche lability" and "niche conservatism," can provide useful heuristics, but overinterpretation risks narrowing research questions and reinforcing historical assumptions. A parallel example comes from thermal biology: Early generalizations that all lizards are careful behavioral thermoregulators, rooted in Cowles and Bogert's desert-focused studies (97, 98), persisted for decades despite evidence that many tropical species are thermal conformers. To the extent that some phenomena may generate more scientific interest than others, the adoption of certain terms may also bias research efforts; for example, the number of papers since 1990 with the term "adaptive radiation" exceeds those with "nonadaptive radiation" nearly 100-fold (72,300 vs. 940; accessed on Google Scholar May 28, 2026).

Discovering the factors that prompt and stymie evolution will continue to be of perennial interest in biology. Our results encourage a more axis-specific perspective on the biodiversity continuum. Morphological stasis has long posed an evolutionary paradox (11): Woodland salamanders suggest that this paradox may, in some cases, be partly one of perspective. Signatures of lability and conservatism can differ among niche and trait dimensions, with divergence in one aspect imposing stasis in another (99). These links can arise through ecological coupling among niche axes, as well as through functional integration and trade-offs among traits. For example, in the southern gray-cheeked salamander (*Plethodon metcalfi*), reduced water loss is associated with lower metabolic rate, consistent with a trade-off in which limiting moisture loss also constrains gas exchange (94). An integrative approach encourages us to ask why certain dimensions are labile and others more rigid and to identify the potential "vectors and tensions" (100) that link these contrasting phenomena. Applied across numerous biological systems, we would be better poised to understand why the signatures of lability and conservatism vary across space, traits, and lineages. In woodland salamanders, physiological diversification was likely enabled by two functional features: Direct development, which freed the clade from the aquatic realm, and lunglessness, which lowered energetic costs (29, 101). Both features provided access to a wide range of terrestrial microclimatic niches in temperate North America, where dispersal is often limited and environmental conditions vary at fine spatial scales (75, 102, 103). In other words, the same terrestriality that likely limited morphological diversity may have also unlocked access to physiological diversity.

We close with a quote from the late David Wake (12), who spent many decades unraveling the complexities of plethodontid salamander evolution, and whose many discoveries helped inspire this work. "Multidimensional, hierarchical approaches to problems in evolution within taxa will be increasingly necessary as the knowledge base continues to grow. Not only morphology and ecology but also disparate areas including physiology, development, and behavior benefit from such perspectives. The challenge for the future, in an era of steadily increasing specialization and technical and analytical sophistication, is to see the whole in relation to all the parts."

Methods

Study Species and Study Sites. We captured 277 salamanders from 30 *Plethodon* species (5 to 17 adults per species) between August 2021 and May 2024 (*SI Appendix*, Fig. S11). At each capture point we recorded microclimate (air and soil temperature, relative humidity, wind speed, dew point, elevation). We transported salamanders to Yale University, where they were acclimated ≥3 wk under controlled laboratory conditions before physiological assays. Prior work on plethodontids has shown that season and laboratory acclimation can induce plastic shifts in physiological traits (93, 94, 104). The large phylogenetic and geographic scope of this work logistically required sampling salamanders at different times of year and induced some variation in time spent in the lab prior to experiments. To evaluate whether the timing of trials influenced physiological trait estimates, we tested the effects of days spent in captivity (*Days in Lab*) and the calendar date of measurement (*Day of Year*) on species mean skin resistance to water loss, metabolic rate, and thermal traits. We fitted phylogenetic generalized least squares models using nlme::gls() with a Brownian-motion phylogenetic correlation structure implemented with ape::corBrownian() (105, 106). All the extended methods for laboratory husbandry, the experimental procedures, and collection permits can be found in the *SI Appendix*.

Measuring Thermal Physiological Traits. We quantified three thermal traits: critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), and selected temperature (T_{sel}). CT_{min} and CT_{max} are the lower and upper thermal limits, respectively, for locomotor function during continuous 0.5°C min^{-1} cooling or heating ramps (107, 108). T_{sel} was obtained by allowing individuals to shuttle freely for 2.5 h in a 4 to 32 °C thermal gradient; body surface temperature was logged every 5 min with a type K thermocouple connected to a digital thermometer (HH806AU, Omega Engineering) (109). T_{sel} was calculated as the mean of the central 50% of observations (110). See *SI Appendix* for detailed methods and *SI Appendix*, Table S6 for a summary of trait variation among species.

Measuring Skin Resistance to Water Loss. We quantified skin resistance to water loss (r_i, s cm^{-1}) from evaporative water loss rates obtained with a flow-through respirometry system (Sable Systems). Converting water loss to skin resistance accounts for body size and biophysical conditions (temperature, humidity, airflow) by standardizing the vapor-pressure gradient, allowing comparisons across treatments and ensuring differences reflect physiological variation rather than biophysical confounds. Each salamander was tested at three ecologically relevant body temperatures (13, 17, and 23 °C) and two vapor-pressure deficits (VPDs; 0.50 and 0.70 kPa) in randomized order. After ~25 min acclimation, we measured evaporative water loss at three time points during each temperature–VPD trial and used the lowest stable reading to calculate the water loss rate and skin resistance r_i (42, 111). Trials showing movement artifacts or excretion events (<3% of measurements) were rerun after ≥1 wk or excluded. See *SI Appendix* for detailed methods and *SI Appendix*, Tables S7–S8 for a summary of trait variation among species.

Metabolic Rate. We used the same flow-through respirometry setup to measure resting metabolic rate (MR), modified to include a CO_2 analyzer. Individuals were tested at 13, 17, and 23 °C under a fixed VPD of 0.50 kPa. After ~25 min acclimation, we recorded three replicate CO_2 measurements per individual and used the lowest stable reading to calculate MR. Flow rate (30 to 45 mL/min) and chamber volume were adjusted by body size to enhance signal sensitivity and ensure adequate air turnover. See *SI Appendix* for detailed methods and *SI Appendix*, Table S9 for a summary of trait variation among species.

Morphological Data. After completing physiological experiments, salamanders were euthanized with MS-222 in accordance with approved Yale University IACUC protocol (protocol #2022-20297). We then micro-CT scanned three individuals per species using a Nikon XT H 225 scanner (Nikon Metrology) at Yale's Chemical and Biophysical Instrumentation Center. Linear measurements of limb bone lengths (humerus, ulna, femur, tibia), trunk size (distance between the axilla and groin), and snout–vent length (SVL, anterior cranium to pelvic girdle) were measured in VGStudio 2024 using the caliper tool (35). We also segmented crania and extracted 3D mesh files for geometric morphometric analysis (112). Using Checkpoint (Stratovan Corp), we placed 92 homologous landmarks (112) (defined in *SI Appendix*, Table S10) and extracted 3D landmark data. General Procrustes Analysis and principal component analysis were performed using geomorph::gm.prcomp() to quantify variation in cranial shape (113–115).

***Plethodon* Phylogeny.** To perform phylogenetic comparative analyses, we used a previously published time-calibrated species tree, which includes all extant *Plethodon* species and was inferred using anchored hybrid enrichment loci, weighted ASTRAL species tree inference, and fossil-calibrated divergence dating in PAML's MCMCTree (78). The tree was pruned using ape::drop.tip() (106). For species with multiple sampled populations in the phylogeny, we retained the population geographically closest to the representative tip in the phylogeny. The weighted ASTRAL tree produces a single species-tree topology rather than a posterior set, but node support is high (most nodes show >95% local posterior probability) (78). Our evolutionary-rate analyses were also robust to an alternative phylogeny with reduced taxon sampling (i.e., excluding species lacking representation in the newer phylogeny) (*SI Appendix*, Fig. S12). For all subsequent comparative analyses, traits were log-transformed where appropriate. We accounted for body size by calculating residuals against mass (for physiological traits) and SVL (for morphological traits) except in fitted models where mass or SVL was included directly as a covariate. Using this tree, we also calculated phylogenetic signal for each trait using Blomberg's K (116) and Pagel's λ (117).

Assessing the Relationship Between Phenotypic and Climatic Variation. To examine how physiological traits vary with environmental conditions, we extracted bioclimatic data from *WorldClim* (v 2.1) (118), which provides climate data for global terrestrial areas based on historical weather observations from 1970 to 2000. Since our capture localities represent a small, uneven subset of each species' distribution (119), we used habitat suitability models developed by the USGS Gap Analysis Project (120) to represent the predicted geographic range of each species. For each species, we calculated the mean value of each of the 19 bioclimatic variables across its range using the ~1 km^2 resolution dataset to capture fine-scale environmental variation. We ran phylogenetic generalized least squares using nlme::gls(), with a Brownian-motion phylogenetic correlation structure implemented with ape::corBrownian(), to quantify the relationship between environmental variables (field and bioclimatic data) and phenotypic traits (105, 106). As an additional analysis, we repeated these regressions using the full individual-level dataset to assess robustness to within-species variation and measurement error, while accounting for phylogeny and weighting observations by trait-specific measurement error (121); full model-selection results are reported in *SI Appendix*, Tables S11–S13. Separately, to test for correlated evolution among traits, we fitted bivariate Brownian-motion models using mvMORPH::mvBM() (122) for all trait pairs with sufficient species overlap. For each model, we extracted the evolutionary variance–covariance matrix (Σ) and converted it to an evolutionary correlation matrix to obtain the pairwise evolutionary correlation (r) between traits.

Disparity Through Time and Estimation of Adaptive Optima. We calculated trait DTT across each multivariate trait set (including hydric, metabolic, thermal, and linear morphological traits) with geiger::dtt() (123) using 1,500 Brownian motion (BM) simulations as a null model. Using l1ou::estimate_shift_configuration() (v1.43), we fitted multioptima Ornstein–Uhlenbeck (OU) models in which lineages could experience discrete shifts in the optimal trait value (θ), so that different parts of the tree evolved toward different expected trait means rather than a single optimum (124). The method uses lasso (ℓ1) regularization to efficiently generate candidate shift configurations and then selects a parsimonious configuration using an information criterion (e.g., pBIC), which helps limit overfitting. We used these analyses to compare tempo and mode between physiological and morphological trait sets.

Estimating and Comparing Rates of Trait Evolution. To evaluate how each physiological and morphological trait evolved across the *Plethodon* phylogeny, we used a univariate relaxed Brownian motion model employed in RevBayes (125). This model permits the rate to vary among branches (and consequently through time). We ran MCMC for 1,000,000 generations, discarding the first 10% as burn-in. We assessed convergence and mixing of the chains by visually inspecting trace plots and ensuring effective sample sizes (ESS) >200 using Tracer v1.7 (126). To compare the rates of evolution between different suites of traits (physiological versus morphological), we also used a multivariate relaxed BM model that further accounts for the correlated evolution of the continuous traits and permits each trait to have its own rate (127). For this analysis, we ran the MCMC for 2,000,000 generations, also discarding the first 10% as burn-in. We included hydric, metabolic, thermal, and linear morphological traits in our analyses. We excluded cranial morphology summarized as principal component scores to avoid treating data-derived PC axes as comparative traits and instead focused on traits with direct units and clear biological interpretation (128). All continuous traits were log-transformed to improve normality and stabilize variances, and the residuals of physiological traits against body mass were used to account for allometric scaling (129). These transformations are standard in comparative analyses and allowed us to preserve biologically meaningful differences among traits while minimizing the influence of scale. To further evaluate whether differences in variance could contribute to rate differences, we quantified trait variance using KCV. As a unitless metric that is invariant to unit changes, KCV enables comparisons of relative dispersion across traits measured on different scales (130). We also quantified within-species dispersion from the full individual-level dataset and tested whether trait-specific σ^2 covaried with within-species variance, providing a conservative check on potential inflation of rate estimates by within-species variation or measurement error (*SI Appendix*, Table S15).

Data, Materials, and Software Availability. Phenotypic trait data, environmental data, and RStudio and RevBayes scripts have been deposited in Yale Dataverse (131).

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