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Scaling between macro- to microscale climatic data reveals strong phylogenetic inertia in niche evolution in plethodontid salamanders

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Macroclimatic niches are indirect and potentially inadequate predictors of the realized environmental conditions that many species experience. Consequently, analyses of niche evolution based on macroclimatic data alone may incompletely represent the evolutionary dynamics of species niches. Yet, understanding how an organisms' climatic (Grinnellian) niche responds to changing macroclimatic conditions is of vital importance for predicting their potential response to global change. In this study, we integrate microclimatic and macroclimatic data across 26 species of plethodontid salamanders to portray the relationship between microclimatic niche evolution in response to changing macroclimate. We demonstrate stronger phylogenetic signal in microclimatic niche variables than at the macroclimatic scale. Even so, we find that the microclimatic niche tracks climatic changes at the macroscale, but with a phylogenetic lag at million-year timescales. We hypothesize that behavioral tracking of the microclimatic niche over space and phenology generates the lag: salamanders preferentially select microclimates similar to their ancestral conditions rather than adapting with changes in physiology. We demonstrate that macroclimatic variables are weak predictors of niche evolution and that incorporating spatial scale into analyses of niche evolution is critical for predicting responses to climate change.

KEY WORDS: Behavior, climate, ecology, evolution, microclimate, niche evolution .

Niche evolution is central to the ecological dynamics of speciation, community assembly, and species distributions (Chase and Leibold 2002; Ackerly et al. 2006; Stephens and Wiens 2009; Wiens et al. 2010; Emery et al. 2012). The retention of climatic niches over macroevolutionary timescales, a pattern known as "niche conservatism," is widespread (Wiens and Graham 2005; Pearman et al. 2008; Wiens et al. 2010; Kozak and Wiens 2010a). Niche conservatism has been implicated in various phenomena such as the latitudinal diversity gradient (Buckley et al. 2010) and historical patterns of extinction (Hawkins et al. 2006). However, such conservatism is paradoxical given that recent theory and numerous empirical studies demonstrated that adaptation in response to environmental change is often swift (Hendry and Kinnison 1999; Hairston et al. 2005). Thus, the pervasiveness of niche conservatism illuminates a contradiction between microevolutionary processes and macroevolutionary patterns (Hansen and Martins 1996; Schluter 2000; Uyeda et al. 2011). A variety of mechanisms have been suggested to reconcile this paradox including stabilizing selection, genetic constraints, niche-tracking, Red Queen effects, and processes invoking hierarchical dynamics of metapopulations and scale dependency (Hansen and Martins 1996; Hansen and Houle 2004; Voje et al. 2015). Explanations based on the evolutionary differences between populations and species have gained traction as explanations for stasis in recent

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years (Jablonski 2000; Glor et al. 2003; Rolland et al. 2018), but remain largely untested. As a consequence, such explanations remain subject to the criticisms of Hansen and Houle (2004), which are that "all of the proposed mechanisms for preserving optima [niches] are ultimately based on shifting the problem elsewhere." To understand the underpinnings of niche evolution, we argue that it is essential to conduct a quantitative examination of the dynamics of niche evolution across spatial scales.

In addition to issues of scale, most studies fail to consider the role that organisms, especially through their behaviors, play in shaping patterns of niche evolution (Odling-Smee et al. 1996; Ackerly 2003; Pelletier et al. 2009). Behavior influences the magnitude and direction of natural selection experienced by individuals, thus functioning as a major pacemaker for niche evolution (Huey et al. 2003). Regulatory behaviors serve to shield organisms from selection and slow evolution, a phenomenon known as "behavioral inertia" or the "Bogert effect" (Bogert 1949; Bartholomew 1964; Wake et al. 1983; Brandon 1988; Huey et al. 2003; Muñoz and Losos 2018; Salazar et al. 2019). The combination of stabilizing selection and behavioral regulation can result in niche conservatism through retention of phenotypic traits and environmental preferences over long evolutionary timescales (Ackerly 2003). In contrast, organisms may use conditions to which they are not preadapted, or be behaviorally precluded from utilizing such conditions (Muñoz et al. 2014; Muñoz and Bodensteiner 2019), thus exposing themselves to directional natural selection and resulting in niche evolution ("behavioral drive"; Mayr 1963; Bateson 1988).

Despite the potential for behavioral data to enrich our understanding of niche evolution, the vast majority of studies on climatic niche evolution are conducted at the level of macroclimate ($\geq$1 km^2), which far exceeds the microclimatic scale at which many organisms interact with their environments (Suggitt et al. 2011; Farallo and Miles 2016; Farallo et al. 2018). In addition, macroclimatic data are based on mean values derived from long-term time series (months or years) that are extrapolated from data collected from nearby weather stations. Local variation in habitat structure and topography induces microclimate heterogeneity, and this variation cannot be fully captured by downscaling regional climate layers (Suggitt et al. 2011; Farallo and Miles 2016).

Here, we use salamander species from the family Plethodontidae (Order: Caudata) to empirically compare niche evolution across spatial scales and assess the role of behavior in shaping distinct evolutionary patterns. We focus on the Grinnellian niche, which includes the habitats and behaviors required for an organism to persist in an environment (Grinnell 1917). Plethodontids are a species-rich group of salamanders that often have small home ranges (<25 m^2) (Merchant 1972; Kleeberger and Werner 1982; Mathis 1991; Marvin 1998), making microscale habitat structure and climate of primary importance to their existence. These species are lungless and rely on cutaneous respiration for gas exchange. Hence, plethodontids require moist conditions to maintain adequate vapor pressure deficits (VPD), which underscores that specific microhabitat conditions are crucial for metabolic processes (Spight 1968; Spotila 1972; Gatz et al. 1975; Wells 2007; Riddell and Sears 2015; Riddell et al. 2017). Previous research using macroclimatic data has suggested that niche conservatism underlies patterns of high species diversity in this clade (Kozak and Wiens 2006, 2010b). Given the small home ranges (Merchant 1972; Kleeberger and Werner 1982; Mathis 1991; Marvin 1998), restricted habitat use (Welsh and Droege 2001; Petranka and Smith 2005), and narrow physiological requirements (Spotila 1972; Bernardo and Spotila 2006; Markle and Kozak 2018) characteristic of salamanders, macroevolutionary patterns of niche conservatism may be enhanced or eroded when examined at shallower spatial scales.

In this study, we address three core questions. First, to what extent do micro- and macroclimatic variables display phylogenetic signal across plethodontid salamanders, and how do signal estimates compare across these two scales? The data used to address this first question were collected from 1 m^2 plots and 1 km^2 resolution climate models. Second, to what extent can differences in phylogenetic signal across scales can be explained by behavior? To address this question, we compare environmental conditions between random habitat surveys and direct points of capture (which applies the filter of salamander behavior, since individuals may actively select the microhabitat conditions in which they were found). Finally, to what extent does the evolution of the microscale niche respond to changes in macroscale niche across plethodontids? Since macroclimate affects the frequency and abundance of microclimatic conditions, we predict that, given sufficient time, the optimal relationship between these micro- and macroscale variables will be isometric (slope = 1). However, behavioral inertia may slow adaptation and weaken the relationship observed in phylogenetic regressions. To answer these questions, we compare the evolutionary dynamics of the micro- and macroclimatic niche from 26 species of salamanders and incorporate field observations of behavioral habitat use. We consider how behavior impacts patterns of niche evolution, and how these patterns relate to the debate surrounding the role of niche conservatism in ecology and evolution.

Materials and Methods

OVERALL SAMPLING STRATEGY

We surveyed 26 salamander species from 63 field sites throughout Ohio, West Virginia, Virginia, North Carolina, and Tennessee (Supporting Information Fig. S1). Data collection occurred

between May 2012 and April 2015. Surveys were conducted between the months of May and October of each year and between the hours 0715–0025. During our surveys, we collected data on salamander microhabitat use based on 1 m^2 plots centered at the point of capture (described in *Salamander Microhabitat Use Surveys* below). We also conducted time constraint surveys for salamander presence in an area where they are expected to occur, followed by microhabitat data collection at selected random points within the search area (described in *Time constraint and Random Microhabitat Surveys* below). All salamanders were observed and handled in compliance with the Institutional Animal Care and Use Committee of Ohio University (Protocol no.: 13-L-012).

SALAMANDER MICROHABITAT USE SURVEYS

Our surveys were designed to assess microhabitat characteristics at sites based on historical occurrence records of plethodontid species. In total, we sampled microhabitat characteristics for salamanders from 56 of 63 sites. At each site, we searched haphazardly for salamanders. Once we observed a salamander, we established a 1 m^2 capture plot (295 plots), centered at the location of the individual. If additional salamanders were found within an established plot they were also included. In each plot, we recorded air temperature, soil temperature, soil moisture, relative humidity, and leaf litter depth. Air temperature (±0.5°C) and relative humidity (±3%) were measured using a Kestrel® 3500 weather meter and digital psychrometer (Nielsen-Kellerman Co.). As relative humidity is given as a percentage, we used a logit transformation in subsequent statistical analyses. We measured soil temperature using either a ThermaPlus thermocouple or an infrared thermometer (IRT) with a high sensitivity probe (±0.5°C; Thermoworks Inc.) and soil moisture using a HydroSense II (±3%; Campbell Scientific Inc.). We measured leaf litter depth (mm) using a ruler. The number of points within each plot used to estimate microclimate varied depending on whether or not the salamander was moving when it was first detected. If the salamander was moving, then we recorded soil temperature and moisture from five points (center, and each of the four vertices) and used the mean for statistical analyses. If, in contrast, the salamander was under a cover object, we recorded microhabitat data only at the point of capture.

TIME CONSTRAINT AND RANDOM MICROHABITAT SURVEYS

To determine whether salamander microhabitat use (measured above) differed from local available microhabitats, we also conducted time constraint surveys and random habitat surveys at 63 sites (Supporting Information Fig. S1). These 63 sites included all 56 sites from which we sampled salamanders collected from our 1 m^2 plots (see above), as well as seven additional sites. We conducted one person-hour time constraint surveys at our field sites from above (Supporting Information Fig. S1). During each survey, we searched sites for plethodontid salamanders by sifting through leaf litter and turning over cover objects. Surveys were conducted during the day (117 surveys) when salamanders are inactive and often under cover objects, as well as at night (89 surveys) when they are active at the surface. We recorded the same microhabitat data described above from approximately 10 random points. We selected points by starting at the search area center point and used a random compass bearing (1–360°) generated with the "sample" function in R environment for statistical computing, version 3.5.0 (R Core Team 2018). We then moved 10 meters in the chosen direction and repeated the procedure until 10 points were completed. Multiple surveys were conducted at each site ($N = 206$ total surveys; mean = 3.3 surveys per site), which resulted in a mean of 34 randomly selected points per site.

MACROCLIMATE DATA

To estimate macroclimatic measurements, we used the GPS coordinates for each salamander found in our microhabitat use surveys ($N = 413$) to extract monthly temperature and precipitation data at a 1 km^2 resolution (30 arc-seconds) from the WorldClim v2.0 database (Fick and Hijmans 2017). Because our data did not encompass the complete geographic range of most of our focal species, we also extracted temperature and precipitation data for a comprehensive set of museum records ($N = 7{,}589$) used by Kozak and Wiens (2010a).

PHYLOGENETIC TREE

We used the phylogenetic tree of amphibians from Pyron and Wiens (2011) that was time calibrated by Eastman et al. (2013). We pruned the phylogenetic tree to the 26 species we encountered during our surveys. The mean value and SE were calculated for each of the micro- and macroclimatic variables for each species. We were unable to calculate the SE of macroclimatic variables for salamander species that were found at a single site during our study (two species out of 26). For these taxa, the SE was estimated by taking the median SD of all other species.

PHYLOGENETIC ASSOCIATION BETWEEN MICRO- AND MACROCLIMATIC NICHES

The following analyses were all conducted using the R environment for statistical computing, version 3.5.0 (R Core Team 2018). First, we compared values for phylogenetic signal between salamander microclimate and macroclimate. In particular, we compared signal for (1) microclimatic variables from the random habitat surveys, (2) microclimatic data reflecting salamander habitat choice (salamander observations), and (3) macroclimatic data based on WorldClim variables extracted from collection localities.

We evaluated two metrics of overall phylogenetic signal, Pagel's λ and phylogenetic half-life estimated from a single-optimum Ornstein–Uhlenbeck (OU) model (Pagel and Harvey 1989; Hansen et al. 2008). To estimate Pagel's λ, we used the function "phylosig" from the phytools package (Revell 2012), and conducted a likelihood ratio test for statistical significance. Measures of SE were included in our calculations of Pagel's λ. If $\lambda > 0.5$ and we could reject $\lambda = 0$ ($P < 0.05$) for a given variable, we considered there to be significant phylogenetic signal. We also calculated the phylogenetic half-life ($t_{1/2}$) of a single optimum OU model using the package mvMORPH (Clavel et al. 2015), which provides an alternative measure of overall phylogenetic signal that can be interpreted as the amount of time required for a lineage to get halfway to its phenotypic optimum (Hansen et al. 2008; Münkemüller et al. 2015). A short $t_{1/2}$ (relative to the length of the tree) means the phylogenetic signal degrades at a rapid pace (e.g., if the half-life is near the age of the youngest split in the tree). In contrast, a $t_{1/2}$ approaching or exceeding the length of the tree converges on a Brownian Motion model of trait evolution (i.e., high phylogenetic signal). We visualized trait evolution on the phylogeny using the function "contMap" from the package phytools (Revell 2012), which uses a Brownian Motion model to reconstruct values at the nodes. We included estimates of measurement error in all analyses. Sample size and power for our phylogenetic analyses of signal are dependent on number of species, not number of individuals, provided that measurement error is included in the analysis (Ives et al. 2007; Hansen et al. 2008).

Intuitively, there should be a strong association between the frequency of available microclimatic niches and the prevailing macroclimate (Holmes and Nelson Dingle 1965). Thus, we hypothesized that species should respond to changing macroclimate by adapting their microclimatic preferences to match the most frequently occurring conditions. However, salamanders may resist such adaptive changes by using an alternative path: specifically, organisms may behaviorally modify their microclimate use to experience conditions within their ancestral preferences. This phenomenon, known as behavioral inertia (Huey et al. 2003), can slow adaptation to changing macroclimatic conditions, and result in a phylogenetic lag in adaptation between the microscale niche and the macroscale climate. To test this hypothesis, we used the SLOUCH model of Hansen et al. (2008), which simultaneously estimates an "evolutionary regression" and an "optimal regression" using an OU modeling framework. The evolutionary regression describes the observed relationship between predictors (macroclimate) and response variables (microclimate) while accounting for phylogeny. The estimated "optimal regression" describes the relationship predicted under an OU model if all taxa exhibited full adaptation of their microclimate to the observed macroclimate predictor values. A difference between the evolutionary and optimal regression slopes is supported when the phylogenetic half-life of the model is bounded away from 0, which is interpreted as phylogenetic inertia (Hansen et al. 2008). In other words, the model accounts for the macroclimatic history of species using their present-day values, and jointly models the evolutionary history of both micro- and macroclimate. This approach assumes that species existing in a given macroclimate for long periods of time are expected to more closely conform to the optimal regression, whereas species that have recently invaded a new macroclimates may instead exhibit high levels of phylogenetic inertia and residual maladaptation (Hansen et al. 2008).

We fit two sets of models with different response variables that exhibited phylogenetic signal (soil temperature and relative humidity) against macroclimate covariates (macroclimatic mean annual temperature and mean annual precipitation, respectively) as randomly evolving predictor variables under a model of Brownian Motion (Hansen et al. 2008). It should be noted we analyzed temperature and moisture variables separately, rather than calculating VPD, for two reasons. First, it allows for a more relevant comparison between the micro- and macroclimatic variables, and second, different combinations of temperature and relative humidity values can result in the same VPD. Therefore, using VPD would erode some of the behavioral choices made by salamanders (e.g., one species may use cooler drier conditions whereas another uses warmer and wetter conditions to maintain similar VPDs). Models were fit using Maximum Likelihood and likelihood surfaces were visualized using SLOUCH's grid search routine to evaluate whether phylogenetic half-life could be bounded away from 0 (i.e., if the evolutionary and optimal regressions are distinct). To incorporate observational error, we included the measurement variance (SE) of the mean values in our models. We compared models that included macroscale temperature, precipitation, and both temperature and precipitation as covariates using AICc values. For microclimatic temperature models, the units for both the response and predictor are same, and we therefore predicted that the slope of micro- and macroscale temperature for the optimal regressions should be 1 given an expected linear relationship between increasing climatic temperatures on microclimatic temperatures. In other words, we tested the null hypothesis that microclimatic variables exhibit a perfect tracking of macroclimatic variables. As precipitation and relative humidity are measured using different units, we also expect a positive slope, but not equal to 1.

ACCOUNTING FOR POTENTIAL SAMPLING BIAS IN MICROCLIMATE DATA

Because our design includes 26 species from multiples sites that we sampled over several years and across a wide geographic range, our sampling of microclimatic data was necessarily limited by the near impossibility of true random sampling of

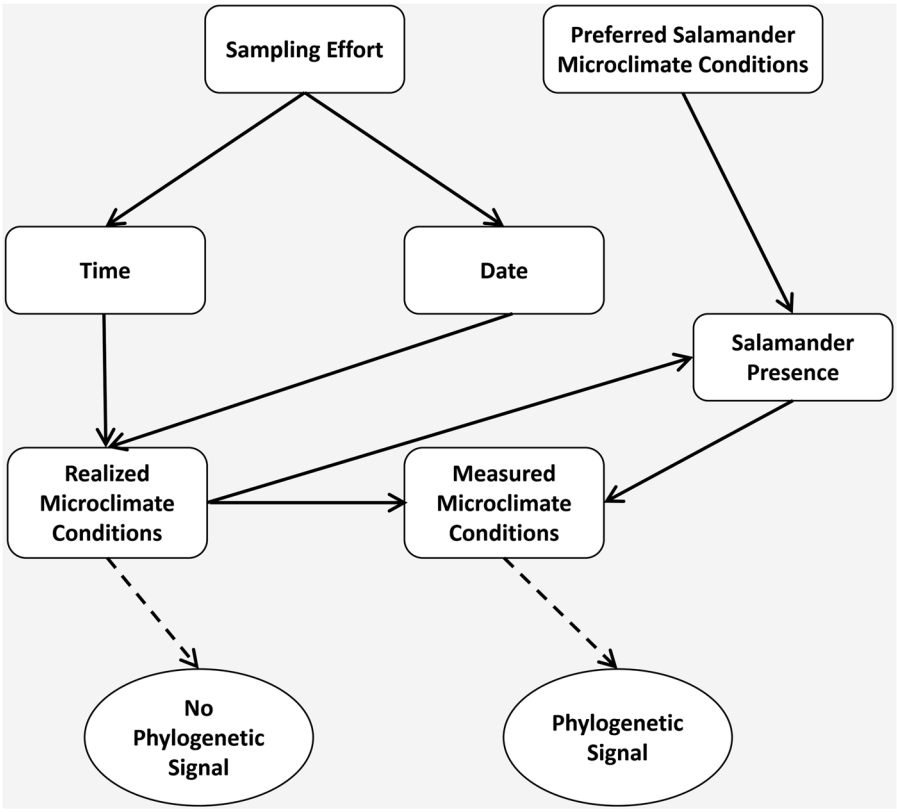

Figure 1. Graphical representation of our causal model describing the effect of sampling date and time on phylogenetic signal. Phylogenetic signal that emerges only after conditioning on salamander presence indicates that behavioral selection of microhabitat by salamanders biases microhabitat variables.

localities over the course of the study. Given that date and time almost certainly affect measured microclimatic temperature and precipitation, we gathered and analyzed additional data to ensure nonrandom sampling effort did not introduce spurious estimates of phylogenetic signal. To test whether sampling effort (e.g., date, time, month) introduced a bias in our estimates of microclimate use, we modeled relative humidity and temperature using simple trigonometric random effects models fixed to annual and diurnal cycles (microclimate variable $\sim$ sin(2π*time) + cos(2π*time) + sin(2π*date) + cos(2π*date) + (1|year)+ (1|site)). We used this model along with our sampling dates and times to generate climatic data that would be expected given our sampling scheme, devoid of salamander biology, and estimated phylogenetic signal from these fitted data.

As expected, date and time of day are strong predictors of observed microclimatic conditions, and much to our initial surprise, microclimatic variables predicted using a cyclical climate model and the empirical day and times of salamander collection themselves have strong phylogenetic signal. We found it unlikely that our idiosyncratic sampling of localities somehow perfectly tracked the phylogeny of plethodontids. Instead, we considered the possibility that time of collection may not represent a confounding variable that should be "controlled for" (e.g., examining residual variation controlling date and time), but may in fact be a mediating variable by which behavioral preferences are expressed. To test this, we followed the logic of our conceptual causal model (Fig. 1) to identify the key prediction that could distinguish among these possibilities (Pearl 2009). Specifically, the model suggests that we should observe increasing phylogenetic signal only when conditioned on salamander presence, but no phylogenetic signal in microclimatic variables when microclimatic data from all surveyed dates and times are included (regardless of whether salamanders were found at sites, Fig. 1). To test this prediction, we estimated species means for soil temperature and relative humidity (the two variables with highest levels of phylogenetic signal) from four subsets of data: (1) all random sites for habitat surveys where a given species was detected as present over the course of the study, (2) all random sites for habitat surveys where a given species was detected on the same date, (3) all random sites for habitat surveys where a given species was detected on the same date, weighted by the approximate number of salamanders found on that date, and (4) direct site data from our 1 m^2 plots associated with each salamander capture. These four subsets differ only by increasing levels of behavioral

Table 1. Phylogenetic signal estimates for micro- and macroclimate variables.

Variable	λ	*p*-value	$t_{1/2}$	ΔAICc
		Microclimate		
Air temperature	0	1.00	9.49	1.24
Relative humidity	0.85	0.07	16.07	3.25
Soil temperature	0.74	0.04	12.61	3.45
Soil moisture	0	1.00	5.36	−1.95
Leaf litter depth	0	1.00	0.26	0.00
		Macroclimate (this study)		
Temperature	0	1.00	3.02	0.30
Precipitation	0	1.00	0.61	−2.57
		Macroclimate (Kozak and Wiens 2010b)		
Temperature	0	1.00	4.64	0.44
Precipitation	0	1.00	0.15	−2.57

We use two metrics of phylogenetic signal, Pagel's lambda (λ) and phylogenetic half-life ($t_{1/2}$). Phylogenetic half-life was estimated from a single-optimum OU model. We provide the *P*-value based on a likelihood ratio test against the alternative hypothesis of λ = 0. We provide the ΔAICc comparing the OU model and a white noise model (e.g., no phylogenetic signal) with higher values indicating stronger support for the OU model.

filtering from our initial sample of random site data across a haphazard schedule of survey times. In other words, if phylogenetic signal only emerges in microclimatic data after conditioning on salamander presence, then it would suggest that date and time are mediating variables by which salamander microclimatic preferences are expressed (and salamander behavior is the mechanism by which such a mediating variable could be controlled). For each subset, we tested phylogenetic signal across all species sampled during the study and included SEs calculated for each subset.

As described in the Results section below, this analysis validated our causal model (Fig. 1) and indicates that controlling for date and time in our other analyses would be inappropriate, as this would control for the exact mechanism salamanders use to attain their climatic preference (i.e., initiating activity on days when preferred climatic conditions are available).

Results

Over the course of this study, we encountered 2,914 individual salamanders from 26 species of plethodontids (Supporting Information Tables S1, S2). We detected strong and significant phylogenetic signal as measured by both Pagel's λ and phylogenetic half-life in microclimate for soil temperature and marginally significant ($P = 0.07$) phylogenetic signal for microclimate relative humidity (Table 1; Fig. 2D). This result is in striking contrast to the absence of evidence for phylogenetic signal in the macroscale temperature and precipitation variables (Table 1; Supporting Information Fig. S2). Phylogenetic half-life for soil temperature ($t_{1/2} = 12.61$ million years) and relative humidity ($t_{1/2} = 16.07$ million years) had moderate half-life values, whereas all macroclimate variables had very low half-life values (Table 1). The lack of phylogenetic signal in macroclimate was consistent when using both the smaller dataset based on salamander capture localities during this study and using an expanded data set based on museum records (Kozak and Wiens 2010a). Therefore, we proceeded to conduct the remaining analyses using data based on our direct capture localities. Furthermore, analysis of the survey and random habitat data, which were collected at the microscale ignoring salamander behavior, revealed no evidence of niche conservatism (Table 2). Thus, species exhibit substantially more phylogenetic signal when climatic conditions are measured at a restricted geographic scale specific to site and time of capture.

It is nearly impossible to sample wide geographic areas and multiple species randomly over the course of a single year. Thus, some bias could have been introduced by the irregular schedule for sampling in the field. We compared our primary phylogenetic analyses of salamander microclimatic data against the same analyses using microclimatic data for all days on which surveys were conducted, regardless of whether salamanders were collected on that day (both using 1 m^2 plots and random habitat data from surveys). If similar levels of phylogenetic signal exist when all the survey locations are included in the analysis, then we can conclude that bias in sampling dates and times due to idiosyncratic scheduling decisions biased our results. However, if phylogenetic signal only emerges after conditioning based on salamander presence, then we can conclude that salamander behavior is the only mechanism by which such a difference could be observed (Fig. 1). We found that date and time influenced soil temperature, but not relative humidity (Fig. 2). Consistent with our hypothesis of behavior driving phylogenetic inertia, we observed a noticeable increase in phylogenetic signal with

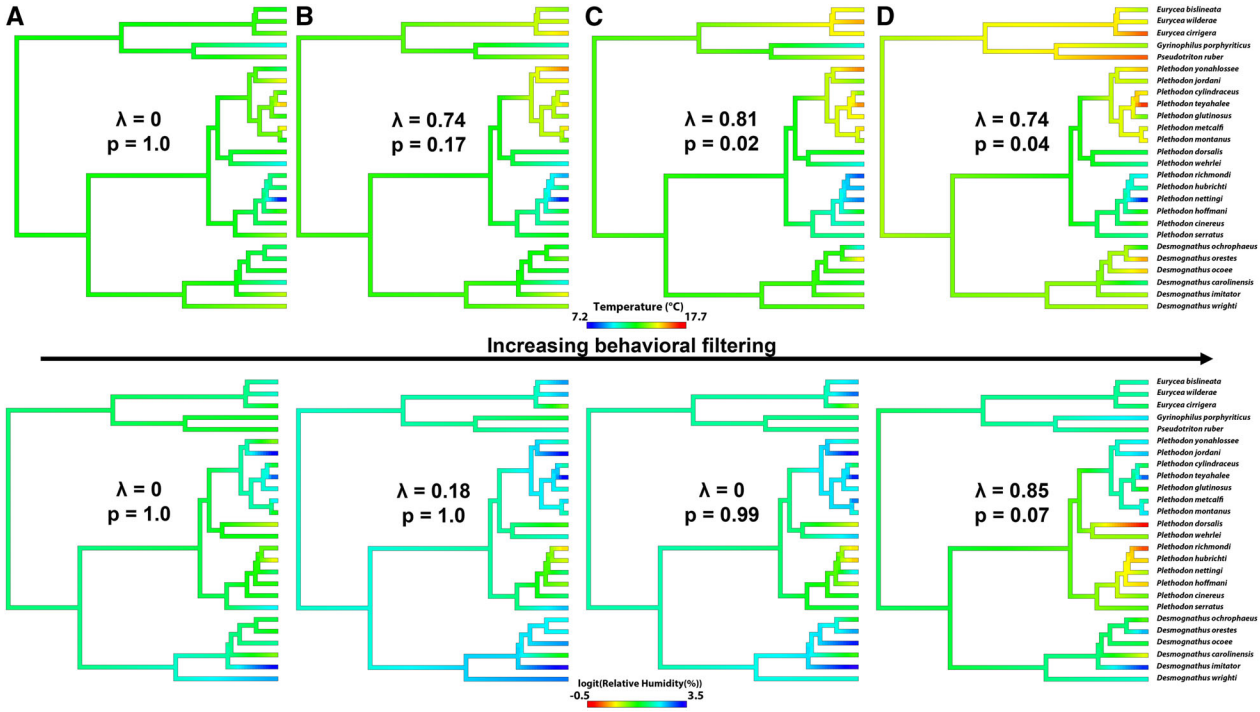

Figure 2. Mean species values of soil temperature (top) and relative humidity (bottom) calculated based on four subsets of the data: (A) all random sites for habitat surveys where a given species was detected as present over the course of the study, (B) all random sites for habitat surveys where a given species was detected on the same date, (C) all random sites for habitat surveys where a given species was detected on the same date, weighted by the number of salamanders found on that date, and (D) direct site data from 1 m^2 plots associated with each salamander capture. Values were mapped onto the time-calibrated ultrametric phylogenetic tree (tree length = 82.30 million years). The corresponding Pagel's lambda (λ) value and its significance from a randomization test is provided for each data subset.

Table 2. Comparison of the models tested for the response variables soil temperature and relative humidity.

			Evolutionary		Optimal			
Predictor variables	$t_{\frac{1}{2}}$	v_y	Intercept (±SE)	Slope (±SE)	Intercept (±SE)	Slope (±SE)	R^2 (%)	AICc
Soil temperature								
temp	**25.42**	**3.11**	**6.13 ± 2.90**	**0.62 ± 0.21**	**5.95 ± 2.84**	**1.05 ± 0.35**	**22.45**	**112.93**
prec	12.18	3.88	13.44 ± 2.40	0.00497 ± 0.0185	13.44 ± 2.41	0.00626 ± 0.0237	0.26	119.17
temp * prec	13.66	2.90	9.06 ± 2.52	0.00315 ± 0.0015	9.06 ± 2.52	0.00412 ± 0.0020	14.08	115.18
Relative humidity								5
temp	14.32	0.65	3.60 ± 1.31	−0.15 ± 0.10	3.63 ± 1.29	−0.20 ± 0.13	6.97	75.4
prec	**7.61**	**0.41**	**−1.18 ± 0.96**	**0.0219 ± 0.0074**	**−1.18 ± 0.96**	**0.0252 ± 0.0086**	**24.37**	**71.46**
temp * prec	12.99	0.64	−0.14 ± 1.18	0.0011 ± 0.0007	−0.15 ± 1.18	0.0014 ± 0.0009	8.20	75.22

Predictor variables include temperature (temp) and precipitation (prec) based on WorldClim 2 data (1 km^2) extracted from our study site locations. Values for phylogenetic half-life ($t_{1/2}$) in millions of years (tree length = 82.30 million years) and Stationary Variance (v_y) are included in the table. Intercept (±SE) and slope (±SE) are provided for both the bias-corrected evolutionary and optimal regressions. Values in bold indicate the model chosen based on comparison of AICc values.

increasing levels of behavioral filtering when the data are conditioned on salamander presence and abundance (Fig. 2). Date and time have a clear relationship with soil temperatures used by salamanders, but phylogenetic signal was not present when all surveys were included. Rather, signal only emerged after conditioning on salamander activity. For this reason we did not incorporate temporal components into our remaining analyses, as including these variables would control for the very mechanism

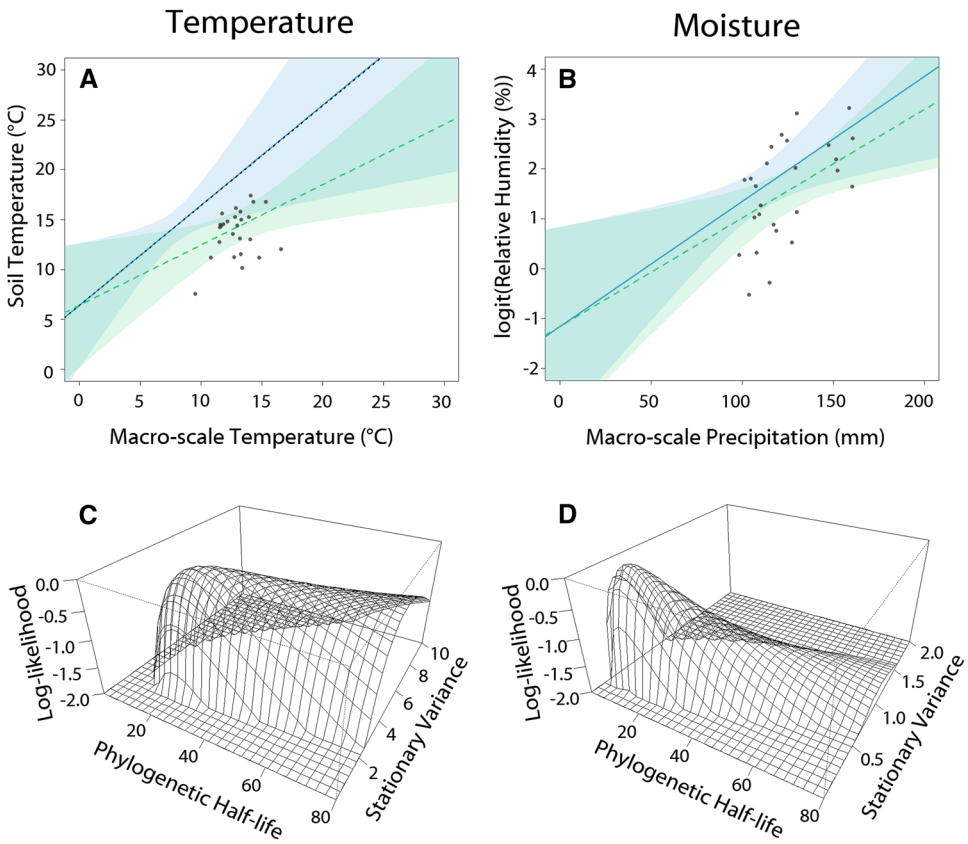

Figure 3. **Optimal (solid blue) and evolutionary (dashed green) regressions of mean soil temperature on macroscale temperature (A) and relative humidity on macroscale precipitation (B). 95% confidence bands are included around both the optimal (blue) and evolutionary (green) regression lines. We also include a line on the temperature panel (A) with a slope of one with the same intercept as the optimal regression to demonstrate the slope of the optimal regression is close to 1 (dashed black). Likelihood support regions for estimates of the phylogenetic half-life and stationary variance (niche width) for soil temperature (C) and logit-scaled relative humidity (D) as predictors of macroscale temperature and precipitation, respectively. Phylogenetic half-life is bounded away from 0 for temperature but not relative humidity. We interpret this as significant evidence of a difference between evolutionary and optimal regressions in temperature, but not in relative humidity. Macroscale temperature and precipitation are modeled as a Brownian Motion process, with microscale response variables evolving as an Ornstein–Uhlenbeck process tracking the macroscale niche. The phylogenetic half-life is presented in millions of years.**

by which the salamander's microclimatic niche is expressed, namely by behavioral preference (Pearl 2009).

We tested whether we could detect a response between microclimatic variables (1 m^2 plot data) and macroclimatic change using the R package SLOUCH (Hansen et al. 2008). We estimated an optimal regression between micro- and macroscale temperatures that closely aligned with the null hypothesis that the slope would be equal to 1, albeit with wide confidence intervals (Slope ± SE; 1.05 ± 0.35; $R^2 = 0.22$; Fig. 3A; Table 2). This suggests that, given enough time, microclimatic variables evolve to match macroclimatic niches. However, the slope of jointly estimated evolutionary regression is shallower than the slope from the optimal regression (Slope ± SE; 0.62 ± 0.21; Table 2). This results from an evolutionary lag, which we estimated to have a phylogenetic half-life of 25.42 million years (Fig. 3C). This estimate is bounded away from 0 (Fig. 3C) and indicates that evolving toward the predicted 1:1 relationship within a lineage requires a time scale in the millions of years and supports a significant difference between the optimal and evolutionary regression. Including precipitation did not substantially impact the model. The model that included only soil temperature had the highest support (Table 2).

Relative humidity used by salamanders showed a positive relationship with macroscale precipitation, which explained 24% of the variance and was our best fit model (Fig. 3B, Table 2). Similar to our microscale temperature regressions, we found a slightly shallower slope for the relative humidity evolutionary regression (Slope ± SE; 0.0219 ± 0.0074) compared to the optimal regression (Slope ± SE; 0.0252 ± 0.0086). However, the lag is less pronounced, with a phylogenetic half-life of 7.61

million years and high likelihood support regions that include short half-lives (Fig. 3D, Table 2). Thus, we do not find evidence of strong phylogenetic inertia for relative humidity and precipitation and cannot reject the possibility that the evolutionary and optimal regressions are the same.

Discussion

The importance of microhabitat use in structuring patterns of niche evolution has been recognized for decades (Ebersole 1985; Huey et al. 2003; Buckley et al. 2015; Moyer-Horner et al. 2015; Muñoz and Losos 2018). Yet, most studies of niche evolution are conducted at the level of the macroclimate ($\geq$1 km^2), which may not represent the ecologically relevant climatic data for many, if not most, organisms. This discrepancy will be especially true for salamanders, which are small-bodied, terrestrial, and possess small home ranges (Kozak and Wiens 2006, 2010b; Wooten et al. 2013). We expected macroevolutionary change in the microscale niche will eventually respond to changing macroclimate, but such responses may be buffered by regulatory behaviors, such as habitat selection or other biological factors. Our findings support this hypothesis: whereas we find strong evidence of phylogenetic signal in the microclimatic niche, there is almost no phylogenetic signal for the macroclimatic niche (Fig. 2; Table 1). The difference in phylogenetic signal across scales indicates that naive usage of macroclimatic data in niche analyses may be inadequate to describe niche evolution in these taxa. As a consequence, spatial scale is of critical importance when making ecological assessments rooted in evolutionary history.

The short phylogenetic half-life in macroclimatic variables compared to the evolutionary history of the group (Table 1) appears to contrast with studies that underscore niche conservatism in Appalachian salamanders (Kozak et al. 2006; Kozak and Wiens 2006, 2010b). Our goal is not to contradict this finding, but rather to illustrate that niche "conservatism" and "lability" are best interpreted as relative terms. For example, at the level of macroclimate, our data suggest that temperature, which exhibited a longer phylogenetic half-life ($t_{1/2}$ = 3.02 million years), is relatively more conserved than relative humidity ($t_{1/2}$ = 0.61 million years). In contrast, all microclimate variables (except for leaf litter depth) appear more conserved than their macroclimatic counterparts. We emphasize that interpretations of niche evolution are both trait- and scale-dependent (Ackerly 2009). Likewise, we argue that the key issue is not to dichotomize species' niches as either "conserved" or "labile," but rather to determine why phylogenetic inertia varies among geographic scales and among traits (Muñoz and Losos 2018).

Several factors could account for the observed differences in phylogenetic signal between micro- and macroclimatic variables. One reason is that the downscaling of 1 km^2 variables may not be linear and entails more complex relationships. For example, downscaling variation in mountainous terrain may result in poor spatial resolution when grid cells encompass considerable topographic complexity arising from elevational variation (Sears et al. 2011; Wang et al. 2016). While poor spatial downscaling could contribute to disparity in evolutionary patterns, we found that microclimatic variables from random survey plots scaled in the expected linear relationship with macroclimatic variables (Fig. 2). Thus, analysis of micro- and macroclimatic niches resulted in similar evolutionary patterns when analyzed in the same way. Specifically, similar patterns were detected when the data were collected at sites where salamanders exist, but without considering their activity levels or presence on the day of data collection. Bias introduced by nonrandom sampling cannot explain the correlation between the two types of variables.

We suggest that selectivity in microclimate use by salamanders impedes microclimate niche evolution and is the bridge that links the phylogenetic inertia across macro- and microclimatic scales. Only by incorporating the effects of behavioral microhabitat exploitation do we find that microscale variables exhibited phylogenetic clustering. The phylogenetic signal becomes even stronger when the data are weighted by the relative abundance of salamanders found at a site on a given day. In contrast, we detected no phylogenetic signal for random habitat points. We suggest that these data are consistent with the Bogert effect: salamanders prefer and select microhabitats matching their physiological optima, resulting in a phylogenetic lag with respect to macroclimate. As a consequence, behavioral preferences buffer salamanders against directional selection on physiology, resulting in lower turnover in microclimatic preferences (Bogert 1949; Huey et al. 2003). We note that our analyses indirectly inferred thermo- and hydroregulation by comparing presence points to random habitat points. An explicit behavioral analysis (i.e., through a null approach *sensu* Hertz et al. 1993) can be used as a more robust test of this conclusion. Nevertheless, we note that other factors (besides behavior) may be contributing to lower turnover in microclimate such as biotic interactions and density-dependent effects.

Based on our results, a natural follow-up question might be whether macroscale data can be converted to microscale data by applying an appropriate transformation that incorporates phylogenetic inertia. Although appealing, this idea is limited by phylogenetic and regional scope because the transformation may change with evolving behavior or environmental conditions. For example, the degree to which behavior can buffer local environment varies among traits, lineages, and regions (Huey et al. 2003; Ortega et al. 2016; Farallo et al. 2018; Muñoz and Losos 2018), meaning that one function does not apply in all cases. Furthermore, the effects of behavior at the microscale may sometimes be to enhance, rather than buffer against, physiological

evolution. For example, due to behavioral lability in habitat use, microscale analyses of coexisting species of *Anolis* lizards result in assemblages comprised of closely related species with little ecological overlap, whereas macroscale data would lump these species into similar niches (Losos et al. 2003). Behavior has the power to both reduce or enhance microclimatic niche variation (Huey et al. 2003; Muñoz and Losos 2018); the pressing question is why one process occurs rather than the other in different cases.

Microclimate selection may explain why a phylogenetic lag exists between micro- and macroclimatic scales, but not why salamanders should exhibit physiological conservatism. Mechanistically, one explanation for this inertia is that salamanders are expected to behaviorally track thermal and hydric conditions that minimize vapor pressure deficit (VPD). VPD is the difference between the moisture in the air and the maximum moisture the air can hold before becoming saturated. A low value for VPD indicates more available moisture. Plethodontid salamanders lack lungs so gas exchange occurs through the skin, which requires ambient moisture. Correspondingly, we observed that salamanders occupying warmer microhabitats (i.e., *Desmognathus*, *Eurycea*, Plethodon *glutinosus* group, *Pseudotriton*, and *Gyrinophilus*) also used microhabitats with the highest relative humidity (Fig. 2D). Selecting microhabitats with a high relative humidity at warmer temperatures minimizes water loss because moisture is lost more rapidly at warmer temperatures. In contrast, salamanders in the *Plethodon cinereus* clade occurred in cooler microhabitats and microsites having the lowest relative humidity (Fig. 2D). At lower temperatures, the hydric cost imposed by VPD is reduced. We note that microclimatic niche tracking is not the only compensatory mechanism likely occurring in salamanders. For example, salamanders from the *Plethodon glutinosus* group exhibit local adaptation in thermal physiology such as higher skin resistance to water loss in warmer habitats (Riddell and Sears 2015). Thus, it is likely that microclimate selectivity is accompanied by physiological changes. Patterns of microclimatic evolution have a strong physiological basis, with a clear role for behavior influencing physiology by mediating water loss rates (Riddell et al. 2018).

Microhabitat stability is relevant for understanding the evolutionary history of plethodontid salamanders. Diversity in most taxa tends to peak in tropical regions, but unlike other vertebrate ectotherms, the biodiversity hotspot for plethodontids is in the temperate zone of the eastern United States (Pianka 1966; Gaston and Blackburn 2000; Hillebrand 2004; Kozak 2017). Niche conservatism has featured prominently as a causal mechanism for the latitudinal diversity gradient: due to climatic stability in the tropics over deep time, physiological isolation can contribute to high species richness (Hawkins et al. 2006; but see Buckley et al. 2010). The striking microhabitat niche conservatism by Appalachian salamanders may explain why this lineage is so diverse despite being in an environment characterized by high climatic variability (see Kozak and Wiens 2006; Kozak and Wiens 2010b for a similar argument based on macroclimatic niches). Although macroclimate can exhibit a more rapid turnover, as predicted for temperate lineages (Stevens 1989), behavioral preferences belie deeper-scale patterns of microclimatic stability. When coupled with periods of geographic isolation (e.g., separation on different mountaintops with unsuitable intervening habitat), phylogenetic retention of fine-scale preferences can provide a proximate mechanism for high plethodontid species richness in the Appalachians.

In addition to harboring a disproportionate amount of salamander diversity, the Appalachian Mountains are predicted to experience climate change that will imperil these (and many other) organisms (Milanovich et al. 2010). A major question in predicting species' responses to climate change is whether the tempo of niche evolution can keep pace with anticipated novel climatic conditions. An important corollary of the phylogenetic inertia we observe here is that macroclimate data alone are poor predictors of how species will experience shifting environmental conditions. Far from evolving in response to macroscale shifts in humidity and temperature, we suggest that salamanders are more likely to use behavior to seek out their preferred microhabitats (e.g., move lower in the leaf litter or under larger cover objects for greater environmental buffering) or alter their physiological phenology (e.g., change seasonal activity patterns). These responses have the potential to modulate short-term impacts of climate change, but at the possible cost of slowing adaptation to prevailing conditions. Given the particular importance of VPD in lungless salamanders, rising temperatures will likely impel individuals to search for moister microhabitats (Farallo et al. 2018).

We emphasize that microscale data can and should be incorporated into ecological and evolutionary studies. Microscale climatic data are likely to be evolutionarily decoupled from macroscale climate, because at microscale conditions will be influenced by temporal activity patterns, heterogenous topography, and land cover (Nowakowski et al. 2018). One of the best approaches available to incorporate microscale data will be the use of mechanistic niche models or biophysical models (Kearney and Porter 2004; Kearney and Porter 2009; Monahan 2009; Gifford and Kozak 2012; Peterson et al. 2016; Riddell et al. 2017; Riddell et al. 2018). Physiological data can be used to model species demography and activity to predict where and when species are likely to occur. In short, physiological data combined with microclimatic models can predict potential for population growth and decline (e.g., energy available for reproduction) and therefore highlight where species are likely to maintain or expand their populations. Furthermore, models of microclimates are also available (Kearney et al. 2014a; Kearney et al. 2014b; Lembrechts et al. 2019). Mechanistic niche models based on microclimatic data will allow for detailed predictions

of species distributions at a biologically relevant scale. The data required for these models are often much more challenging to collect than data for traditional macroscale analyses. One solution to this problem will be to build on existing techniques by creating models that incorporate habitat selection data while using macroclimate data to calculate the microclimate conditions. The framework for this is already established with the microclim model (Kearney et al. 2014a), which can estimate microclimate conditions based on macroclimate and terrain data. Current mechanistic models incorporate species-specific physiological data to then estimate key demographic parameters such as predicted hours of activity (Sinervo et al. 2018). However, if microclimate selected is associated with the presence of other species, food availability, or other climatic variables, then current biophysical models may still overlook important factors structuring distributions. Filling in these relevant ecological gaps will be key for making these models more relevant for improving predictions of species' vulnerability to climate change.

AUTHOR CONTRIBUTIONS

VRF and DBM conceived the study. VRF completed field work and collected data. VRF, JCU, and MMM worked on analyses. VRF wrote the first draft of the manuscript and all authors contributed substantially to revisions.

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DATA ARCHIVING

Data used in this study are archived on Dryad (doi: https://doi.org/10.5061/dryad.sf7m0cg2j).

LITERATURE CITED

Ackerly, D. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. Int. J. Plant Sci. 164:S165–S184.

———. 2009. Conservatism and diversification of plant functional traits: evolutionary rates versus phylogenetic signal. Proc. Natl. Acad. Sci. 106:19699–19706.

Ackerly, D. D., D. W. Schwilk, and C. O. Webb. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. Ecology 87:S50–S61.

Bartholomew, G. A. 1964. The roles of physiology and behaviour in the maintenance of homeostasis in the desert environment. Pp. 7–29 In G. M. Hughes, ed. Homeostasis and feedback mechanisms. Cambridge Univ. Press, Cambridge, UK,

Bateson, P. 1988. The active role of behaviour in evolution. Pp. 191–207 *in* M.-W. Ho, and S. W. Fox, eds. Evolutionary processes and metaphors. Wiley, Chichester.

Bernardo, J., and J. R. Spotila. 2006. Physiological constraints on organismal response to global warming: mechanistic insights from clinally varying populations and implications for assessing endangerment. Biol. Lett. 2:135–139.

Bogert, C. M. 1949. Thermoregulation in reptiles, a factor in evolution. Evolution 3:195–211.

Brandon, R. N. 1988. The levels of selection: a hierarchy of interactors In H. C. Plotkin, ed. The role of behavior in evolution. MIT Press, Cambridge, MA.

Buckley, L. B., T. J. Davies, D. D. Ackerly, N. J. B. Kraft, S. P. Harrison, B. L. Anacker, H. V. Cornell, E. I. Damschen, J.-A. Grytnes, B. A. Hawkins, et al. 2010. Phylogeny, niche conservatism and the latitudinal diversity gradient in mammals. Proc. R. Soc. Biol. Sci. Ser. B 277:2131–2138.

Buckley, L. B., J. C. Ehrenberger, and M. J. Angilletta. 2015. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. Funct. Ecol. 29:1038–1047.

Chase, J. M., and M. A. Leibold. 2002. Spatial scale dictates the productivity-biodiversity relationship. Nature 416:427–430.

Clavel, J., G. Escarguel, and G. Merceron. 2015. mvMORPH: an R package for fitting multivariate evolutionary models to morphometric data. Methods Ecol. Evol. 6:1311–1319.

Eastman, J. M., L. J. Harmon, D. C. Tank, and E. Paradis. 2013. Congruification: support for time scaling large phylogenetic trees. Methods Ecol. Evo.l 4:688–691.

Ebersole, J. P. 1985. Niche separation of two damselfish species by aggression and differential microhabitat utilization. Ecology 66: 14–20.

Emery, N. C., E. J. Forrestel, G. Jui, M. S. Park, B. G. Baldwin, and D. D. Ackerly. 2012. Niche evolution across spatial scales: climate and habitat specialization in California *Lasthenia* (*Asteraceae*). Ecology 93:S151–S166.

Farallo, V. R., and D. B. Miles. 2016. The importance of microhabitat: a comparison of two microendemic species of *Plethodon* to the widespread *P. cinereus*. Copeia 104:67–77.

Farallo, V. R., R. Wier, and D. B. Miles. 2018. The Bogert effect revisited: salamander regulatory behaviors are differently constrained by time and space. Ecol. Evol. 8:11522–11532.

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37:4302–4315.

Gaston, K. J., and T. M. Blackburn. 2000. Pattern and process in macroecology. Blackwell Scientific, Oxford, U.K.

Gatz, R. N., E. C. Crawford, and J. Piiper. 1975. Kinetics of inert-gas equilibration in an exclusively skin-breathing salamander, *Desmognathus fuscus*. Respir. Physiol. 24:15–29.

Gifford, M. E., and K. H. Kozak. 2012. Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. Ecography 35:193–203.

Glor, R. E., J. J. Kolbe, R. Powell, A. Larson, and J. B. Losos. 2003. Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). Evolution 57:2383–2397.

Grinnell, J. 1917. The niche-relationships of the California Thrasher. The Auk 34:427–433.

Hairston, N. G., S. P. Ellner, M. A. Geber, T. Yoshida, and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecol. Lett. 8:1114–1127.

Hansen, T. F., and D. Houle. 2004. Evolvability, stabilizing selection, and the problem of stasis. Pp. 130–150 *in* K. P. M. Pigliucci, ed. The evolutionary biology of complex phenotypes. Oxford Univ. Press, Oxford, U.K.

Hansen, T. F., and E. P. Martins. 1996. Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. Evolution 50:1404–1417.

Hansen, T. F., J. Pienaar, and S. H. Orzack. 2008. A comparative method for studying adaptation to a randomly evolving environment. Evolution 62:1965–1977.

Hawkins, B. A., J. A. F. Diniz-Filho, C. A. Jaramillo, and S. A. Soeller. 2006. Post-Eocene climate change, niche conservatism, and the latitudinal diversity gradient of New World birds. J. Biogeogr. 33:770–780.

Hendry, A. P., and M. T. Kinnison. 1999. Perspective: the pace of modern life—measuring rates of contemporary microevolution. Evolution 53:1637–1653.

Hertz, P. E., R. B. Huey, and R. D. Stevenson. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. Am. Nat. 142:796–818.

Hillebrand, H. 2004. On the generality of the latitudinal diversity gradient. Am. Nat. 163:192–211.

Holmes, R. M., and A. Nelson Dingle. 1965. The relationship between the macro- and microclimate. Agr. Meteorol. 2:127–133.

Huey, R. B., P. E. Hertz, and B. Sinervo. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. Am. Nat. 161:357–366.

Ives, A. R., P. E. Midford, and J. T. Garland. 2007. Within-species variation and measurement error in phylogenetic comparative methods. Syst. Biol. 56:252–270.

Jablonski, D. 2000. Micro- and macroevolution: scale and hierarchy in evolutionary biology and paleobiology. Paleobiology 26:15–52.

Kearney, M., and W. Porter. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecol. Lett. 12:334–350.

———. 2004. Mapping the fundamental niche: Physiology, climate, and the distribution of a nocturnal lizard. Ecology 85:3119–3131.

Kearney, M. R., A. P. Isaac, and W. P. Porter. 2014a. microclim: global estimates of hourly microclimate based on long-term monthly climate averages. Sci. Data. 1:140006.

Kearney, M. R., A. Shamakhy, R. Tingley, D. J. Karoly, A. A. Hoffmann, P. R. Briggs, and W. P. Porter. 2014b. Microclimate modelling at macro scales: a test of a general microclimate model integrated with gridded continental-scale soil and weather data. Methods Ecol. Evol. 5:273–286.

Kleeberger, S. R., and J. K. Werner. 1982. Home range and homing behavior of *Plethodon cinereus* in northern Michigan. Copeia 1982:409–415.

Kozak, K. H. 2017. What drives variation in plethodontid salamander species richness over space and time? Herpetologica 73:220–228.

Kozak, K. H., D. W. Weisrock, and A. Larson. 2006. Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: *Plethodon*). Proc. R. Soc. Biol. Sci. Ser. B 273:539–546.

Kozak, K. H., and J. J. Wiens. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. Evolution 60:2604–2621.

———. 2010a. Accelerated rates of climatic-niche evolution underlie rapid species diversification. Ecol. Lett. 13:1378–1389.

———. 2010b. Niche conservatism drives elevational diversity patterns in Appalachian salamanders. Am. Nat. 176:40–54.

Lembrechts, J. J., I. Nijs, and J. Lenoir. 2019. Incorporating microclimate into species distribution models. Ecography 42:1267–1279.

Losos, J. B., M. Leal, R. E. Glor, K. de Queiroz, P. E. Hertz, L. Rodriguez Schettino, A. C. Lara, T. R. Jackman, and A. Larson. 2003. Niche lability in the evolution of a Caribbean lizard community. Nature 424:542–545.

Markle, T. M., and K. H. Kozak. 2018. Low acclimation capacity of narrow-ranging thermal specialists exposes susceptibility to global climate change. Ecol. Evol 8:4644–4656.

Marvin, G. A. 1998. Territorial behavior of the plethodontid salamander *Plethodon kentucki*: influence of habitat structure and population density. Oecologia 114:133–144.

Mathis, A. 1991. Territories of male and female terrestrial salamanders: costs, benefits, and intersexual spatial associations. Oecologia 86:433–440.

Mayr, E. 1963. Animal species and evolution. Belknap, Cambridge, MA.

Merchant, H. 1972. Estimated population size and home range of the salamanders *Plethodon jordani* and *Plethodon glutinosus*. J. Wash. Acad. Sci. 62:248–257.

Milanovich, J. R., W. E. Peterman, N. P. Nibbelink, and J. C. Maerz. 2010. Projected loss of a salamander diversity hotspot as a consequence of projected global climate change. PLoS One 5:e12189.

Monahan, W. B. 2009. A mechanistic niche model for measuring species' distributional responses to seasonal temperature gradients. PLoS One 4:e7921.

Moyer-Horner, L., P. D. Mathewson, G. M. Jones, M. R. Kearney, and W. P. Porter. 2015. Modeling behavioral thermoregulation in a climate change sentinel. Ecol. Evol. 5:S810–S822.

Münkemüller, T., F. C. Boucher, W. Thuiller, and S. Lavergne. 2015. Phylogenetic niche conservatism—common pitfalls and ways forward. Funct. Ecol. 29:627–639.

Muñoz, M. M., and B. L. Bodensteiner. 2019. Janzen's hypothesis meets the Bogert effect: connecting climate variation, thermoregulatory behavior, and rates of physiological evolution. Integr. Organism. Biol. 1: oby002.

Muñoz, M. M., and J. B. Losos. 2018. Thermoregulatory behavior simultaneously promotes and forestalls evolution in a tropical lizard. Am. Nat. 191:E15–E26.

Muñoz, M. M., M. A. Stimola, A. C. Algar, A. Conover, A. J. Rodriguez, M. A. Landestoy, G. S. Bakken, and J. B. Losos. 2014. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. Proc. R. Soc. Biol. Sci. Ser. B 281:9.

Nowakowski, A. J., L. O. Frishkoff, M. Agha, B. D. Todd, and B. R. Scheffers. 2018. Changing thermal landscapes: merging climate science and landscape ecology through thermal biology. Curr. Landsc. Ecol. Rep. 3:57–72.

Odling-Smee, F. J., K. N. Laland, and M. W. Feldman. 1996. Niche construction. Am. Nat. 147:641–648.

Ortega, Z., A. Mencía, and V. Pérez-Mellado. 2016. Behavioral buffering of global warming in a cold-adapted lizard. Ecol. Evol. 6:4582–4590.

Pagel, M. D., and P. H. Harvey. 1989. Comparative methods for examining adaptation depend on evolutionary models. Folia Primatol. 53:203–220.

Pearl, J. 2009. Causal inference in statistics: an overview. Statist. Surv. 3:96–146.

Pearman, P. B., A. Guisan, O. Broennimann, and C. F. Randin. 2008. Niche dynamics in space and time. Trends Ecol. Evol. 23:149–158.

Pelletier, F., D. Garant, and A. P. Hendry. 2009. Eco-evolutionary dynamics. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364:1483–1489.

Peterson, A., M. Papes, and J. Soberón. 2016. Mechanistic and correlative models of ecological niches. Eur. J. Ecol. 1:28–38.

Petranka, J. W., and C. K. Smith. 2005. A functional analysis of streamside habitat use by southern Appalachian salamanders: implications for riparian forest management. For. Ecol. Manag. 210:443–454.

Pianka, E. R. 1966. Latitudinal gradients in species diversity: a review of concepts. Am. Nat. 100:33–46.

Pyron, R. A., and J. J. Wiens. 2011. A large-scale phylogeny of amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Mol. Phylogenet. Evol. 61:543–583.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217–223.

Riddell, E. A., E. K. Apanovitch, J. P. Odom, and M. W. Sears. 2017. Physical calculations of resistance to water loss improve predictions of species range models. Ecol. Monogr. 87:21–33.

Riddell, E. A., J. P. Odom, J. D. Damm, and M. W. Sears. 2018. Plasticity reveals hidden resistance to extinction under climate change in the global hotspot of salamander diversity. Sci. Adv. 4:eaar5471.

Riddell, E. A., and M. W. Sears. 2015. Geographic variation of resistance to water loss within two species of lungless salamanders: implications for activity. Ecosphere 6:1–16.

Rolland, J., D. Silvestro, G. Litsios, L. Faye, and N. Salamin. 2018. Clownfishes evolution below and above the species level. Proc. R. Soc. Biol. Sci. Ser. B 285:9.

Salazar J. C., Castañeda M. R., Londoño G. A., Bodensteiner B. L., Muñoz M. M.. 2019. Physiological evolution during adaptive radiation: A test of the island effect in Anolis lizards. Evolution 73 (6): 1241 –1252. https://doi.org/10.1111/evo.13741.

Schluter, D. 2000. Ecological character displacement in adaptive radiation. Am. Nat. 156:S4–S16.

Sears, M. W., E. Raskin, and M. J. Angilletta, Jr. 2011. The world is not flat: defining relevant thermal landscapes in the context of climate change. Integr. Comp. Biol. 51:666–675.

Sinervo, B., D. B. Miles, Y. Wu, F. R. Méndez-De La Cruz, S. Kirchhof, and Y. Qi. 2018. Climate change, thermal niches, extinction risk and maternal-effect rescue of toad-headed lizards, *Phrynocephalus*, in thermal extremes of the Arabian Peninsula to the Qinghai—Tibetan Plateau. Integr. Zool. 13:450–470.

Spight, T. M. 1968. The water economy of salamanders: evaporative water loss. Physiol. Zool. 41:195–203.

Spotila, J. R. 1972. Role of temperature and water in the ecology of lungless salamanders. Ecol. Monogr. 42:95–125.

Stephens, P. R., and J. J. Wiens. 2009. Bridging the gap between community ecology and historical biogeography: niche conservatism and community structure in emydid turtles. Mol. Ecol. 18:4664–4679.

Stevens, G. C. 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. Am. Nat. 133:240–256.

Suggitt, A. J., P. K. Gillingham, J. K. Hill, B. Huntley, W. E. Kunin, D. B. Roy, and C. D. Thomas. 2011. Habitat microclimates drive fine-scale variation in extreme temperatures. Oikos 120:1–8.

Uyeda, J. C., T. F. Hansen, S. J. Arnold, and J. Pienaar. 2011. The million-year wait for macroevolutionary bursts. Proc. Natl. Acad. Sci. 108:15908–15913.

Voje, K. L., Ø. H. Holen, L. H. Liow, and N. C. Stenseth. 2015. The role of biotic forces in driving macroevolution: beyond the Red Queen. Proc. R. Soc. Biol. Sci. Ser. B 282:20150186–20150186.

Wake, D. B., G. Roth, and M. H. Wake. 1983. On the problem of stasis in organismal evolution. J. Theor. Biol. 101:211–224.

Wang, T., A. Hamann, D. Spittlehouse, and C. Carroll. 2016. Locally downscaled and spatially customizable climate data for historical and future periods for North America. PLoS One 11:e0156720.

Wells, K. D. 2007. The ecology and behavior of amphibians. Univ. of Chicago Press, Chicago.

Welsh, H. H., and S. Droege. 2001. A case for using Plethodontid salamanders for monitoring biodiversity and ecosystem integrity of North American forests. Conserv. Biol. 15:558–569.

Wiens, J. J., D. D. Ackerly, A. P. Allen, B. L. Anacker, L. B. Buckley, H. V. Cornell, E. I. Damschen, T. J. Davies, J.-A. Grytnes, S. P. Harrison, et al. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecol. Lett. 13:1310–1324.

Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Annu. Rev. Ecol. Evol. Syst. 36:519–539.

Wooten, J. A., C. D. Camp, J. R. Combs, E. Dulka, A. Reist, and D. M. Walker. 2013. Re-evaluating niche conservatism versus divergence in the Woodland Salamander genus *Plethodon*: a case study of the parapatric members of the *Plethodon glutinosus* species complex. Can. J. Zool. 91:883–892.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplemental Figure 1. A map of our field sites presented on a digital elevation model.

Supplemental Figure 2. Species mean values of temperature and precipitation extracted from our georeferenced field sites using WorldClim 2 data (Fick and Hijmans 2017), demonstrating the lack of phylogenetic signal in contrast with our microclimatic variables.

Supplemental Table 1. Summary of the species encountered in the 1 m^2 plots, including number of individuals (N) and mean elevation where the species was found during the study.

Supplemental Table 2. Summary of species encountered during our random habitat surveys.