Syst. Biol. 73(5):743–757, 2024

https://doi.org/10.1093/sysbio/syae041
Advance Access Publication August 2, 2024

Adaptive Radiation Without Independent Stages of Trait Evolution in a Group of Caribbean Anoles

Brooke Bodensteiner[1,*], Edward D. Burress[2], and Martha M. Muñoz[1]

[1]*Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect St, New Haven, CT 06511, USA*
[2]*Department of Biological Sciences, University of Alabama, 1325 Hackberry Ln, Tuscaloosa, AL 35401, USA*
[*]*Correspondence to be sent to: Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect St, New Haven, CT 06511, USA; E-mail: brooke.bodensteiner@yale.edu.*

Received 12 July 2023; reviews returned 25 June 2024; accepted 18 July 2024
Associate Editor: Frank Burbrink

Abstract.—Adaptive radiation involves diversification along multiple trait axes, producing phenotypically diverse, species-rich lineages. Theory generally predicts that multi-trait evolution occurs via a "stages" model, with some traits saturating early in a lineage's history, and others diversifying later. Despite its multidimensional nature, however, we know surprisingly little about how different suites of traits evolve during adaptive radiation. Here, we investigated the rate, pattern, and timing of morphological and physiological evolution in the anole lizard adaptive radiation from the Caribbean island of Hispaniola. Rates and patterns of morphological and physiological diversity are largely unaligned, corresponding to independent selective pressures associated with structural and thermal niches. Cold tolerance evolution reflects parapatric divergence across elevation, rather than niche partitioning within communities. Heat tolerance evolution and the preferred temperature evolve more slowly than cold tolerance, reflecting behavioral buffering, particularly in edge-habitat species (a pattern associated with the Bogert effect). In contrast to the nearby island of Puerto Rico, closely related anoles on Hispaniola do not sympatrically partition thermal niche space. Instead, allopatric and parapatric separation across biogeographic and environmental boundaries serves to keep morphologically similar close relatives apart. The phenotypic diversity of this island's adaptive radiation accumulated largely as a by-product of time, with surprisingly few exceptional pulses of trait evolution. A better understanding of the processes that guide multidimensional trait evolution (and nuance therein) will prove key in determining whether the stages model should be considered a common theme of adaptive radiation. [adaptive radiation; *Anolis*; ecomorphology; ecophysiology; trait evolution.]

Adaptive radiation—the rapid proliferation of a single ancestor into several ecologically and phenotypically diverse descendants—is responsible for much of Earth's biodiversity (Morinaga et al. 2023), resulting in some of the most visually spectacular lineages known, like African rift lake cichlids, Hawai'ian honeycreepers, and Caribbean anole lizards (Simpson 1953; Schluter 2000; Losos 2009). Adaptive radiations are often diverse in their morphology, physiology, and behavior, with species carving up trait space along multiple phenotypic axes to limit ecological overlap among close relatives (Pianka 1986; Schluter 2000; Losos et al. 2003; Streelman and Danley 2003; Ackerly et al. 2006; Givnish 2015; Martin and Richards 2019). Despite its central importance for adaptive radiation, it is largely unclear whether multidimensional trait evolution occurs via shared or independent pathways. Different classes of traits like physiology and morphology may diversify in concert if they respond to shared or correlated selective pressures, or if they have similar genetic or developmental constraints (Lande and Arnold 1983; Felsenstein 1988; Armbruster et al. 2014; McGlothlin et al. 2018, 2022). Theory largely predicts, however, that different classes of traits diversify independently and in a sequential manner, reflecting iterative bouts of trait specialization to distinct selective pressures (Streelman and Danley 2003; Gavrilets and Losos 2009; Gillespie et al. 2020). Under this "stages" model of adaptive radiation, ecological overlap among close relatives is limited, in turn permitting the dense species packing often observed in adaptive radiations (e.g., Losos et al. 2003). In replicated adaptive radiations, like African Rift Lake cichlids, diversification via stages is even proposed to occur convergently, implying a deterministic nature to the specific order of trait evolution (Streelman and Danley 2003; Gavrilets and Losos 2009; Ronco et al. 2021).

While this model is conceptually intuitive, in practice, we know surprisingly little about how different suites of traits (e.g., morphology and physiology) diversify during adaptive radiation, and even less about whether they do so sequentially. Sequential radiation implies that selective pressures underpinning phenotypic specialization can be isolated, meaning that selection acts independently on different traits. Nevertheless, there are several factors that could result in shared patterns of evolution during adaptive radiation. A given ecological resource may have several co-varying selective pressures operating in concert: different structural niches, for example, often have unique physical properties and microclimatic properties, and so could simultaneously exert selection on functional morphology, thermal physiology, and hydric physiology (Huey et al. 2003; Muñoz and Losos 2018; Leahy et al. 2021; Alomar et al. 2024; Stroud et al. 2024). Prior work has shown, for example, that high-elevation anoles on the Caribbean island of Hispaniola perch on boulders, in contrast to the arboreal perches preferred by their low-elevation counterparts (Muñoz and Losos 2018). This perch switch at high elevation prompted functional specialization in head and limb dimensions to a saxicolous lifestyle. Boulders are also warmer than tree trunks, and behavioral thermoregulation by selective use of boulders buffered exposure to selection in otherwise cold habitats, in turn limiting thermal specialization across elevation (Muñoz and Losos 2018). This phenomenon—behavioral buffering to limit exposure to selection and phenotypic divergence—is known as the Bogert effect (Bogert 1949; Huey et al. 2003; Muñoz 2022).

Likewise, a given environmental feature or ecological resource may simultaneously impose selection and drive evolution in multiple traits: body size, limb proportions, and thermal physiology, for example, all adaptively evolve in response to environmental temperature (Bergmann 1847; Allen 1877; James 1970; Ashton and Feldman 2003; Muñoz and Losos 2018; McQueen et al. 2022). Moreover, phenotypic traits may be evolutionarily correlated via genetic linkage, functional constraints, or developmental constraints, limiting freedom of evolution (Lande and Arnold 1983; Felsenstein 1988; Wagner and Altenberg 1996; Walker 2007; Armbruster et al. 2014; Muñoz 2019). That different niche axes are uncorrelated and that trait evolution is sequential is more often assumed than directly tested.

An opaque conceptual framework and a paucity of empirical studies leave unclear how multidimensional trait evolution should proceed during adaptive radiation. We propose that a rigorous empirical exploration should involve several considerations. First, one should consider whether selective pressures are likely to be shared or independent among classes of traits. To what extent, for example, is a certain structural niche (e.g., perch type) also a certain microclimatic niche? Next, one should assess whether phenotypic specialization is shared or independent among suites of traits. For example, do species that overlap in morphological dimensions (e.g., ecomorphs) also overlap in physiological dimensions (e.g., thermal specialization)? Lastly, rates of evolution should be uncorrelated among independently evolving traits. Evolutionary rates should decline for traits that saturate early in a radiation's history and accelerate when specialization occurs closer to the present. The predicted order of trait saturation varies among lineages, reflecting system-specific features (discussed in Streelman and Danley 2003; Gavrilets and Losos 2009).

The adaptive radiation of Caribbean *Anolis* lizards, a lineage characterized by high ecomorphological and ecophysiological diversity, presents an ideal study system with which to address these questions. In the Greater Antilles (Cuba, Hispaniola, Jamaica, and Puerto Rico) different morphological specialists, termed "ecomorphs," independently evolved to exploit distinct structural supports, such as twigs, bushes/grasses, and tree trunks (Losos 2009). Caribbean anoles also repeatedly specialized in their ecophysiology to exploit different thermal microhabitats along the sun-shade axis, such as closed-canopy cloud forests and open savannas (Ruibal 1961; Rand 1964; Schwartz and Henderson 1991) and into different thermal macrohabitats across elevational gradients, from sea level to several kilometers above it (Hertz and Huey 1981; Muñoz et al. 2014a). In a classic study, Williams (1972) conceptually stitched these two aspects of anole phenotypic diversity into a single framework, proposing that initial specialization in body size and limb proportions to structural habitat use was followed by physiological specialization to different thermal habitats. Although rarely examined, indirect evidence generally supports this "stages" model of adaptive radiation. Hertz et al. (2013) found that the phylogenetic signal is higher for morphology than for physiology, suggesting that morphological variation saturates earlier in the radiation. On Puerto Rico, sister species in the same ecomorph occupy different thermal habitats (open/edge vs. closed-canopy) and tend to vary in their heat tolerance and the optimal sprinting temperature, suggesting that divergence in morphology is followed by divergence in ecophysiology (Hertz et al. 2013; Gunderson et al. 2018).

Despite many advances, however, previous approaches have had limitations. For example, the "stages" hypothesis has largely centered around within-ecomorph evolution from low- to mid-elevation assemblages. This story, however, is left incomplete by the exclusion of 'unique' species that lack ecomorphological correlates across the Greater Antilles (Losos 2009; Schettino et al. 2010; see Supplementary Material for more detail (**Supplementary Tables S1 and S2**). Likewise, high-montane endemics have not received as thorough a study as low-elevation species, even though diversification into distinct thermal macrohabitats provides a clear substrate for adaptive specialization (Muñoz et al. 2014a; Sunday et al. 2019). Little is known about the association among different ecological niche axes (i.e., the relationship between structural and thermal niches), the corresponding relationship between morphological and physiological evolution, and how evolutionary rates vary through time. Notably, the specific order of trait evolution is predicted to be similar within lineages (Streelman and Danley 2003; Ronco et al. 2021), necessitating inter-island comparisons whether radiation via stages is repeatable among replicate radiations. We, therefore, lack a cohesive, synthetic understanding of multidimensional niche divergence and trait evolution in anoles.

Here, we addressed this gap by investigating the pattern, rate, and timing of morphological and physiological evolution in *Anolis* lizards from Hispaniola. Anoles on this island are especially diverse in structural habitat use, as there are representatives from 6 ecomorphs, as well as several ecomorphologically "unique" species. Hispaniolan anoles are also diverse in the thermal habitats they occupy, with some species utilizing open/edge habitats and others restricted to closed canopy/shaded habitats (Schwartz and Henderson 1991; Henderson and Powell 2009). Hispaniola bears the highest Caribbean peak (>3,000 m.a.s.l.), and elevational distributions vary among anoles, with some species found near sea level, others several kilometers above sea level, and much variation therein (Schwartz 1989; Henderson and Powell 2009; Losos 2009). Focusing on Hispaniolan anoles, we address the following questions: (1) How correlated are structural and thermal niche characteristics? (2) How does phenotypic diversity relate to structural microhabitat use and thermal microhabitat use? (3) How correlated are patterns of morphological and physiological evolution to the thermal macrohabitat (e.g., across elevation)? (4) How do rates of morphological and physiological evolution vary, and do these features evolve independently? (5) Is there a temporal sequence of trait divergence among morphological and physiological traits? Specifically, do morphological traits exhibit an "early burst" and do physiological traits exhibit a "late burst" of evolution (Williams 1972)? Our hypotheses for each of these questions are explicitly detailed in the Supplementary Materials (**Supplementary Table S1**). By addressing these questions, we present a more holistic understanding of the timing and patterns of trait evolution in a classic case of adaptive radiation. We then consider how multitrait evolution shapes ecological overlap among closely related species and discuss the mechanisms underlying the exceptional phenotypic diversity of this island's anole radiation. Lastly, we illustrate how a multidimensional trait perspective enhances our understanding of how adaptive radiation proceeds and discuss whether such diversification should be expected to occur in discrete stages in anole lizards and in other radiations.

Materials and Methods

Study Species and Sites

The Caribbean island of Hispaniola is comprised of two nations, Haiti and the Dominican Republic. Our study focused on 29 anole species collected in the Dominican Republic, representing 69% (29/42 species) of the island's diversity (**Supplementary Tables S2 and S3**). The 29 species in this study include representatives from 6 recognized anole ecomorphs (i.e., trunk-ground, trunk, trunk-crown, twig, grass-bush, and crown-giant) (**Fig. 1**). Ecomorph category provides a proxy for structural habitat use, which reflects perch height, type, diameter, pliability, and texture, among other features (Losos 2009). Our dataset also includes 5 "unique" species, meaning that they have no ecomorphological correlates on the other Caribbean islands (**Supplementary Table S2**). In addition to ecomorphological diversity, Caribbean anoles vary in their relative use of canopy (Sun/shade use), with some species primarily found along forest edges or in open habitats ("open/edge habitat" species) and others found predominantly in closed-canopy, shaded habitats like cloud forest ("closed-canopy" species). Our dataset includes representatives from each category (12 open/edge habitats and 17 closed-canopy species; **Supplementary Table S3** (Schwartz and Henderson 1991; Henderson and Powell 2009)). We discretized canopy use to accommodate our phylogenetic analyses (which require discrete predictors), as it provides strong explanatory power for physiological variation in anoles (Ruibal 1961; Rand 1964; Huey and Webster 1976; Gunderson et al. 2018) and other reptiles (Muñoz et al. 2016). We categorized canopy use based on our own observations in the field, which we supplemented with published resources (Schwartz and Henderson 1991; Henderson and Powell 2009). Hispaniola is topographically complex, with multiple ranges > 2,000 m: our sampling design spans the island's elevational gamut, and includes the island's 2 high-montane endemics, *Anolis armouri* (from the Sierra de Baoruco) and *A. shrevei* (from the Cordillera Central).

Structural and Thermal Habitat Data

Our first goal was to assess the relationships among structural and thermal niche attributes (to address question 1 above). To this end, we gathered new and previously published data for anole perch height and perch diameter, 2 key characteristics of structural niche use (Losos 2009). We measured the thermal microenvironment by estimating the operative temperature (T_e) of different structural niches. T_e is known as the steady-state temperature of an organism in the absence of thermoregulation (Bakken 1992). We measured T_e using copper models with embedded iButton (thermochron model DS1922L-F5#) temperature sensors. To provide an estimate of the thermal microenvironment experienced by lizards, we gathered T_e data across an elevational range (0–2500 m.a.s.l.) and across various structural microhabitats corresponding to different "ecomorph" perches (see Supplementary Methods for more detail). We used the R package ggcorrplot (Kassambara 2022) to visualize the correlation matrix, including correlation coefficients, between our thermal and structural niche variables.

To provide an estimate of the thermal macroenvironment, we focused on mean annual temperature (MAT), the mean of the lowest temperature of the coldest month (MIN), and the mean of the highest temperature of the warmest month (MAX), as these variables provide a general measure of the available thermal conditions in each habitat (Hijmans et al. 2005). We extracted these variables from our sampling localities and the georeferenced sampling localities for Hispaniolan anole species in Algar et al. (2013) from the environmental layers (~1 km^2 resolution) available in the WorldClim database (Hijmans et al. 2005). To account for multiple measurements and (in several cases) multiple study sites per species, we calculated a weighted species' average of each thermal variable.

Morphological Data

We gathered morphological data from Mahler et al. (2010), which includes several traits strongly associated with structural microhabitat use (i.e., ecomorph identity) in Caribbean anoles (Losos 1990; Mahler et al. 2010). The morphological variables included were

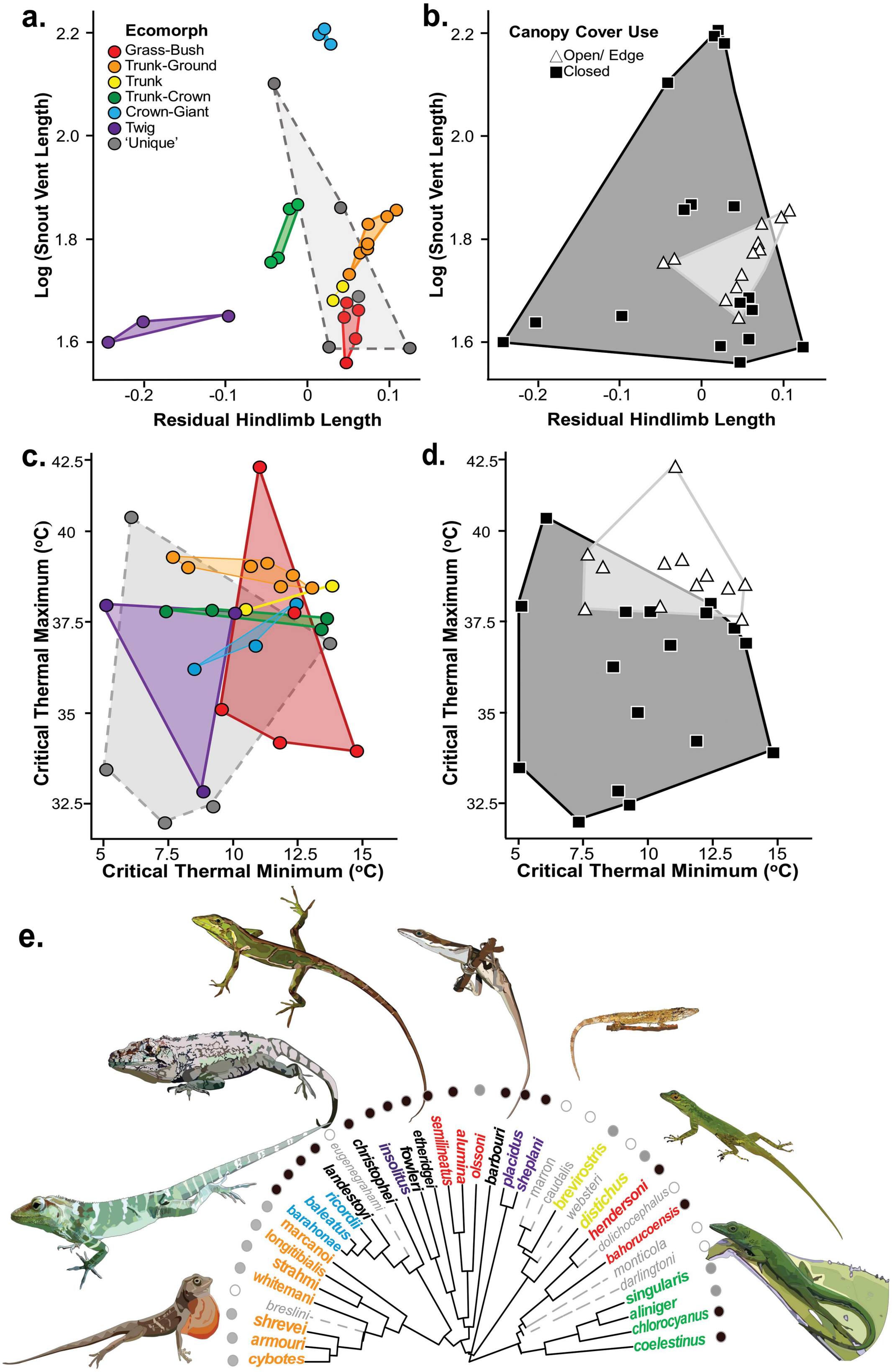

FIGURE 1. Little overlap between morphological and physiological diversity among Hispaniolan anole species. The color of each point indicates the ecomorph type (a, c) and shape corresponds to canopy use (b, d) for each species. Trait space for species of the same ecomorph or canopy use class is wrapped with a convex polygon. Phylogeny of Hispaniolan anoles illustrating the species included in this study (solid

body size (measured as the snout-to-vent length or SVL) and hindlimb length (the sum of femur length, tibia length, length of metatarsus IV, length of hindtoe IV) (Supplementary **Fig. S1**; Supplementary **Table S3**). Mahler et al. (2010) reported species' means for each of these traits (mean = 8 individuals/species). Limb traits were phylogenetically size corrected by regressing log-transformed hindlimb length against log-transformed SVL using the phyl.resid function in the phytools (Revell 2012) R package.

Physiological Data

To investigate physiological evolution in Hispaniolan anoles, we focused on 3 key traits: the critical thermal minimum (CT_{min}), the critical thermal maximum (CT_{max}), and the preferred temperature (T_{pref}). CT_{min} and CT_{max} describe the lower and upper limits, respectively, for locomotor function and are widely used for estimating the thermal limits of performance in ectotherms (Spellerberg 1972; Lutterschmidt and Hutchison 1997). The preferred temperature refers to the average of the central 50% of body temperatures measured in lizards that have been placed in a thermal gradient and allowed to choose where to sit (i.e., in the absence of environmental or ecological constraints) (Huey 1982; Hertz et al. 1993).

We measured thermal traits from 22 Hispaniolan *Anolis* species from May-July 2018, March 2020, and July 2022 (Supplementary **Table S3**). Critical thermal limits and thermal preference were also gathered for seven species from our previously published data (Muñoz et al. 2014a; Muñoz and Bodensteiner 2019) (Supplementary **Figs S2–S3**; Supplementary **Table S3**). Briefly, we captured adult lizards from the wild using a lasso made from fishing line or dental floss that we attached to an extensible panfish pole. Following capture, lizards were individually housed and given a 24-h rest period in an insulated Styrofoam cooler. To measure the core temperature during the tolerance experiments, a temperature probe (Omega, Type T, 40-gauge) was placed ~1 cm into the cloaca of each lizard and secured with medical tape around the base of the tail. The temperature probe was attached to a handheld digital thermometer (Omega, HH806AU), which continuously measured temperature with ± 0.3 °C accuracy.

We estimated the preferred body temperature (T_{pref}) by placing lizards in a laboratory thermal gradient during the lizards' active hours (Taylor et al. 2021). The preferred body temperature is calculated by taking the mean of the central 50% of body temperatures (Huey 1982; Hertz et al. 1993; Taylor et al. 2021) from lizards that have been placed in a thermal gradient and allowed to choose where to spend time in the absence of ecological constraints (e.g., predators). The thermal preference arenas consisted of a box with 8 identical lanes (91 cm × 15 cm × 14 cm). We laid sand over reptile heat cables (150 W; Zoo Med) in an air-conditioned room, creating a stable gradient ranging from 20 °C at one end to 40 °C at the other. Before experiments began, we inserted a thermocouple (Type T, 40-gauge, Omega) ~1 cm into the lizard's cloaca and secured it with medical tape. The thermocouple probe was connected to a digital temperature logger (HH806AU; Omega). Lizards acclimated to the arena for 30 min prior to the experiment. The temperature logger then recorded the core body temperature every 5 min for 3 h. Animals were given a 24-h rest period at room temperature (~25 °C) in individual containers within a large cooler before thermal tolerance trials.

Prior to CT_{min} measurement, lizards were placed in a plastic Tupperware® container, where they could move freely as they acclimated to room temperature. The container was then placed into a Styrofoam cooler layered with crushed ice, where the lizard was cooled by ~1 °C/minute. At 15 °C, the lizard was flipped onto its back and stimulated to right itself by prodding the base of the tail and thighs with blunt tweezers. This was repeated every 0.5 °C change in temperature until the lizard was unable to right itself after 15 s. We defined CT_{min} as the temperature at which the lizard failed to right itself. Animals were then given a 24-h rest period at room temperature (~25 °C) in individual Tupperware® containers within a large cooler before CT_{max} trials. Lizards were warmed with a 100-watt light bulb suspended approximately 30 cm above the Tupperware® container. We increased core body temperature at a rate of 1 °C/min. We began flipping lizards when they began to cool through panting (i.e., the panting threshold (Hertz et al. 1979)) or when their core temperature reached 30 °C. Following the flipping procedure described above, the temperature at which the lizard was unable to right itself after the allotted time was recorded as their CT_{max}.

Anolis Phylogeny

We used the ultrametric maximum clade credibility (MCC) mtDNA phylogenetic tree for Caribbean anoles from Mahler et al. (2010, 2013). Briefly, chronograms were estimated using a partitioned Bayesian analysis of a 6-gene fragment of mtDNA (~1,500 bp). There is uncertainty in the absolute timing of anole diversification in the Caribbean (Losos 2009): following Mahler et al. (2013), we used a tree scaled to a crown age of 50Ma.

black branches) and unsampled species (dashed gray branches). Ecomorph is specified by color as follows: red="grass-bush"; orange="trunk-ground"; yellow="trunk"; green="trunk-crown"; blue="crown-giant"; purple="twig." "Unique" species are denoted in gray. Note that "unique" species do not form a true "group" in the sense of ecomorphs, so they are bounded with a dashed line. Canopy use is specified by circle color as follows: black = closed-canopy; gray = open/edge habitat; white = data deficient. The species art going clockwise from left to right shows *Anolis marcanoi, A. ricordii, A. landestoyi, A. fowleri, A. alumina, A. placidus, A. distichus*, and *A. chlorocyanus*. In the trait space plots (a–d) each dot corresponds to a different species. Anole drawings were provided by S. Rometsch.

Using the R package phytools (Revell 2012), we grafted *A. landestoyi* (a recently described Hispaniolan species) onto the phylogeny following Mahler et al. (2016), and then pruned the tree to the 29 species included in this study. All analyses were conducted in the R environment (R Core Team 2021).

Comparing Phenotypic Diversity With Respect To Structural Habitat Use and Canopy Use

To investigate question 2, we were interested in comparing how morphological and physiological diversity is partitioned among the 6 ecomorphs, among non-ecomorph "unique" species, and according to canopy use ("closed canopy habitat" and 'open/edge habitat'). To account for collinearity among the 3 physiological traits, we reduced the dimensionality of the physiological data with pPCA using the phyl.pca function in the phytools package (Revell 2012), and obtained the correlation matrix using lambda. We retained pPC1 and pPC2 to visualize phylotraitspace, as these axes collectively explained 91% of the variance in the data (Supplementary **Tables S4–S5**). Based on this analysis, CT_{max} and T_{pref} primarily loaded with PC1 and CT_{min} loaded with PC2. Decades of research demonstrated the clustering of same-ecomorph anoles in morphological trait space (Losos 2009; Mahler et al. 2013), so the visualization of ecomorphology is confirmatory. If structural niche use is associated with physiological specialization, then we would expect ecomorphs to also cluster in physiological trait space. Similarly, if the thermal microhabitat niche (canopy use category) is associated with morphological specialization, then we would expect species within the same canopy use category to also cluster in morphological trait space.

For a subset of 22 species, we gathered data relating to perch height and perch diameter. To investigate the degree to which phenotypic variation varies according to structural microhabitat use, we ran PGLS to investigate the relationship between structural habitat use and our traits of interest (Supplementary **Table S6**). Analyses were performed using the pgls function in the caper R package (Orme et al. 2013). To tackle question one, we examined the effects of elevation, perch height, perch diameter, and the interaction between perch height and diameter.

Investigating Phenotypic Adaptation to the Thermal Niche

Next, we examined how phenotypic traits respond to both the thermal macroenvironment (site-level trends in temperature) and thermal microenvironment (captured by canopy use category and operative environmental temperatures (T_e) collected across elevational gradients and structural habitat use).

To investigate the evolutionary relationships between morphological and physiological traits and the thermal macroenvironment (question 3), we used stochastic linear Ornstein-Uhlenbeck (OU) models in the SLOUCH framework (Hansen et al. 2008). This approach estimates the adaptation of phenotypic traits to an evolutionary optimum in response to a randomly changing predictor variable (Hansen et al. 2008). This method simultaneously estimates an "evolutionary regression" and an "optimal regression" using an OU modeling framework. The evolutionary regression describes the observed relationship between a predictor variable (i.e., MAT, MIN, and MAX) and response variables (physiological and morphological traits) while accounting for phylogeny. By contrast, the "optimal regression" describes the relationship predicted under an OU model if all taxa exhibited complete adaptation of their phenotypic trait of interest to the thermal environment. The evolutionary regression slope will deviate significantly from the optimal regression slope when the phylogenetic half-life ($t_{1/2}$) of the model is bounded away from zero, indicating phylogenetic inertia in trait adaptation to the predictor variable (Hansen et al. 2008). Phylogenetic half-life ($t_{1/2}$) describes the time it takes to evolve halfway to an optimum and provides a measure of phylogenetic signal in the residuals (Hansen et al. 2008; Münkemüller et al. 2015). A short $t_{1/2}$ (relative to total tree length) indicates that the phylogenetic signal rapidly degrades and that adaptation to the optimal value occurs quickly (i.e., near the youngest splits in the tree). Longer $t_{1/2}$ (approaching or exceeding total tree length), by contrast, indicates that trait evolution converges on a Brownian motion-like process. The model also estimates stationary variance (v_y), which describes the variance in the optimal trait value, with greater v_y suggesting that taxa explore a broader range of trait space about the phenotypic optimum.

We fitted SLOUCH models using maximum likelihood separately for the following traits: critical thermal maximum, critical thermal minimum, preferred temperature, log body size, and residual hindlimb length. We visualized 3D-likelihood surfaces using a grid search routine to estimate if the phylogenetic half-life is bounded away from zero. Deviation of $t_{1/2}$ away from zero occurs when the evolutionary and optimal regressions differ, indicating a temporal lag in adaptive evolution. Lastly, observational error was included as measurement variance (SE) on the mean values used in our models. As patterns were consistent across different thermal macrohabitat predictor variables, we present results for MAT in the main text, and the results for MIN and MAX are given in the Supplementary Material.

Another way we examined our second question of interest was by investigating the relationship between physiological and morphological traits and thermal microhabitat use (canopy use) via phylogenetic ANCOVA, with phenotypic traits (CT_{min}, CT_{max}, T_{pref}, body size, and residual hindlimb length) as the response variables, the categorical fixed effect of canopy (2 levels: "open/edge habitat" or "closed canopy habitat") and the continuous covariate of elevation.

Comparing Rates and Independence of Trait Evolution

We next estimated and compared evolutionary rates among log-transformed traits using RevBayes

(Höhna et al. 2016) to address our fourth question of interest. The different units associated with morphological and physiological traits preclude direct comparisons of their evolutionary rates; nevertheless, we investigated whether differences in scale and the variance of each trait could contribute to any rate differences. To this end, we calculated a measure of variation that is independent of scale (KCV; Lobry et al. 2023). Given that prior work has shown that thermal physiology varies according to canopy use (e.g., Muñoz et al. 2016; Gunderson et al. 2018), we also assessed if the rate of physiological evolution differed between closed-canopy and open-habitat species using a Bayesian, state-dependent, multivariate relaxed Brownian motion model (May and Moore 2020), implemented in RevBayes (Höhna et al. 2016). As continuous characters we used CT_{max}, CT_{min}, and T_{pref} in a multivariate framework. The Markov chain Monte Carlo (MCMC) was run for 1,000,000 generations with 10% burnin. To assess the robustness of the model to different priors, we repeated the MCMC with different priors on the number of rate shifts (i.e., 5, 10, 20, and 25 shifts). To assess if evolutionary rates were dependent (or independent), we estimated tip-rates (i.e., species-specific evolutionary rates) for each trait using a relaxed Brownian motion model of evolution (Burress et al. 2020; May and Moore 2020; Burress and Muñoz 2022). The MCMC was run for 1,000,000 generations with 10% burnin. Since tip rates are not phylogenetically independent, we assessed pairwise correlations using phylogenetic generalized least squares (Revell 2010). To evaluate the possibility that incomplete taxon sampling could result in false positives, we simulated 100 datasets across the full (unpruned) phylogeny using Brownian motion (a constant rate process), then pruned those datasets to our empirical sampling. We then repeated the MuSSCRat analyses using these simulated datasets. We set the prior number of rate shifts to 0. Analyses with BM-simulated data did not elicit false positives that could be explained by incomplete taxon sampling (all PP < 0.6).

Examining the Timing of Trait Evolution

To address question 5 we applied the node height test to assess the changes in the rate of trait evolution through time in the Hispaniolan anole radiation. This test examines the correlation between the absolute magnitude of phylogenetic contrasts with the heights of the nodes at which the contrasts were generated: strong correlations are consistent with either early- or late-bursts of trait evolution, depending on the sign (negative or positive, respectively) of the relationship (Freckleton and Harvey 2006). The height of the node is specified as the absolute distance between the root and the most recent common ancestor of the pair from which the contrast is generated. Analyses were performed using the function nh.test in the package GEIGER (Harmon et al. 2020). The node height test assumes that a trait is evolving under Brownian motion (BM). This assumption of BM could lead to false positives (higher rates toward the present) if a trait evolution follows an OU process. To explore this more deeply, we simulated data using BM and OU across our tree using the OUwie.sim function in OUwie (Beaulieu et al. 2012; Beaulieu and O'Meara 2015). Of 100 simulations using BM we found that 7% had a significant change in evolutionary rate throughout the tree. By contrast, 54% of were significant when modeled using OU, consistent with a high false-positive rate. When we fitted evolutionary models to each of our traits in OUwie, we found that cold tolerance evolves following an OU-like process, and the other traits evolve via BM (Supplementary **Table S13**). Therefore, we only applied the node height test to CT_{max}, T_{pref}, body size, and hindlimb length.

Given the proposed sequence of trait evolution in anoles (Williams 1972; Hertz et al. 2013) and observed patterns on nearby Puerto Rico (Gunderson et al. 2018), we predicted that body size and (perhaps to a lesser extent) relative hindlimb length saturated early in the radiation (negative relationship), and that thermal limits and the preferred temperature saturated closer to the present day (positive relationship). To address these ideas, we examined the relative timing of trait divergence using the divergence order test (Ackerly et al. 2006). This method is a modification of an independent contrasts approach in which the average node age is weighted by the absolute magnitude of the contrast at each node to provide information about relative trait divergence. By using the absolute value of the contrasts for a trait across the phylogeny and the age at each node we calculated the weighted mean age of divergence for each trait. This results in an average age that designates whether large divergences in a trait tended to take place early or late in the group's history. We utilized a bootstrapping procedure to determine the statistical significance of differences in the average age of divergence in traits. We did this by bootstrapping the distribution of ancestral values to calculate the weighted divergence age for each trait and the difference between these values.

RESULTS

Morphological and Physiological Diversity Are Uncorrelated, Reflecting Independence Among Structural and Thermal Niches

Structural and thermal niche features are uncorrelated: distinct structural perches (e.g., "trunk-crown," or 'twig') are not associated with unique thermal properties (Supplementary **Fig. S4**). Structural niches are warmer at low elevation than at high elevation but the magnitude of the effect of elevation does not vary among structural niche types. By contrast, measures of the thermal macrohabitat and thermal microhabitat are strongly correlated (>0.9) in the expected directions: MAT, MIN, MAX, and T_e all decrease with elevation. Structural niche parameters—perch height and perch diameter—are uncorrelated (<0.2; Supplementary **Fig. S4**; Supplementary **Table S6**).

Consistent with independence among structural and thermal niche features, physiological and morphological diversity are likewise uncorrelated. Whereas ecomorphs segregate in morphological trait space, they do not do so in physiological trait space: we observed broad physiological overlap among ecomorphs (**Fig. 1**). Crown-giants, for example, are morphologically distinct but share physiological trait space with grass-bush, trunk-crown, and twig anoles. Correspondingly, we found no relationship between the structural microhabitat and thermal physiology (Supplementary **Table S6**). The unique anoles occupy a wide range of both morphological and physiological trait space. Phylogenetic ANCOVAs examining canopy-cover use impact on morphological traits likewise indicate no compelling relationships: logSVL $F_{1,27} = 0.222$, $P = 0.642$; residual hindlimb length $F_{1,27} = 3.175$; $P = 0.086$.

Variation in Phenotypic Adaptation to the Thermal Niche

Our SLOUCH analyses showed that all physiological traits adapt to the thermal macrohabitat (heat tolerance: Slope ± SE; 0.165 ± 0.084; cold tolerance: Slope ± SE; 0.353 ± 0.094; thermal preference: Slope ± SE; 0.122 ± 0.085; **Fig. 2**; Supplementary **Table S7**). But, cold tolerance does so much more rapidly (lower phylogenetic half-life) (**Fig. 2**; Supplementary **Table S7**). The lag in adaptation is the longest for heat tolerance followed by a relatively shorter, but still notable, evolutionary lag for the preferred temperature (**Fig. 2**; Supplementary **Table S7**). In contrast to physiology, morphological traits are largely unrelated to the thermal environment. Body size responds to mean annual temperature, albeit more weakly than the physiological traits (Slope ± SE; 0.020 ± 0.011; **Fig. 2**; Supplementary **Table S7**). Specifically, cooler environments are associated with evolutionary reductions in body size. Results were comparable using the other thermal predictors (Supplementary **Table S8**).

The relationship between physiology and the thermal microenvironment (canopy use) differs among traits. Whereas cold tolerance decreases with elevation in all species, heat tolerance remains stable across elevation in open/edge habitat species and decreases with elevation in closed-canopy species. Thermal preference remains stable across elevation regardless of canopy use (**Fig. 3**). By contrast, canopy use is a poor predictor of morphology (SVL: $F_{1,27} = 0.714$, $P = 0.791$; residual hindlimb length: $F_{1,27} = 0.102$, $P = 0.321$; Supplementary **Table S9**).

Uneven Pulses and Rates of Phenotypic Evolution Characterize Hispaniolan Anoles

Rates of trait evolution are unequal across the Hispaniolan radiation. Cold tolerance evolution outpaces all other physiological traits, ranging from 6-fold faster than the preferred temperature to 8-fold faster than heat tolerance (**Fig. 4**). CT_{min} trait values are at a smaller scale and have more variance than SVL, T_{pref}, and CT_{max} (**Supplementary Fig. S6**). While we cannot directly compare rate differences between the physiological traits and body size, we can infer that they cannot be fully explained by differences in scale. All pairwise comparisons of trait tip rates were correlated except cold tolerance and heat tolerance as well as cold tolerance and thermal preference (**Supplementary Table S10**). We found no impact of canopy-cover use category on trait-trait correlations of evolutionary rate (**Supplementary Table S11**). We found that physiological evolution is faster in species from closed-canopy habitats than their counterparts using edge/open habitats (**Fig. 4**). This result was consistent across models with different priors (all PP > 0.95). Univariate analyses reveal that this faster rate of physiological evolution in closed-canopy species is driven by CT_{max} and T_{pref} (both PP > 0.95), and not by CT_{min} (PP = 0.401). None of these traits—CT_{max}, T_{pref}, body size, and hindlimb length—exhibited an early or late burst of evolution (**Supplementary Fig. S7**; **Supplementary Table S13**). Body size, residual hindlimb length, and heat tolerance have relatively older divergence ages, whereas the divergence age for cold tolerance and preferred temperature occurred more recently (**Supplementary Table S12**).

DISCUSSION

Morphological and physiological specialization underpins much of Caribbean anole diversity (Ruibal 1961; Rand 1964; Losos 2009), as well as other adaptive radiations (Schluter 2000; Givnish et al. 2009). Theory predicts that multi-trait evolution should unfold via discrete "stages," and that these stages should follow a predictable order (Streelman and Danley 2003; Gavrilets and Losos 2009; Gillespie et al. 2020; Ronco et al. 2021). The proposed stages model for anoles involves morphological specialization evolving prior to physiological specialization (Williams 1972; Hertz et al. 2013). Here, we united several lines of evidence suggesting that evolution via independent stages was a plausible outcome for Hispaniolan anoles. Despite these favorable ingredients, we found limited support for a stages model of adaptive radiation on this island. We unpack these findings below, and then comment on the generalizability and broader utility of the "stages" model in adaptive radiation theory.

Patterns of Physiological and Morphological Evolution Are (mostly) Unaligned

We found that structural and thermal niches are uncorrelated, both within sites and across elevations. High-canopy perches, for example, are thermally comparable to near-ground and grass-bush perches. Certainly, structural niches are cooler at high elevation, but the magnitude of the elevation effect is comparable

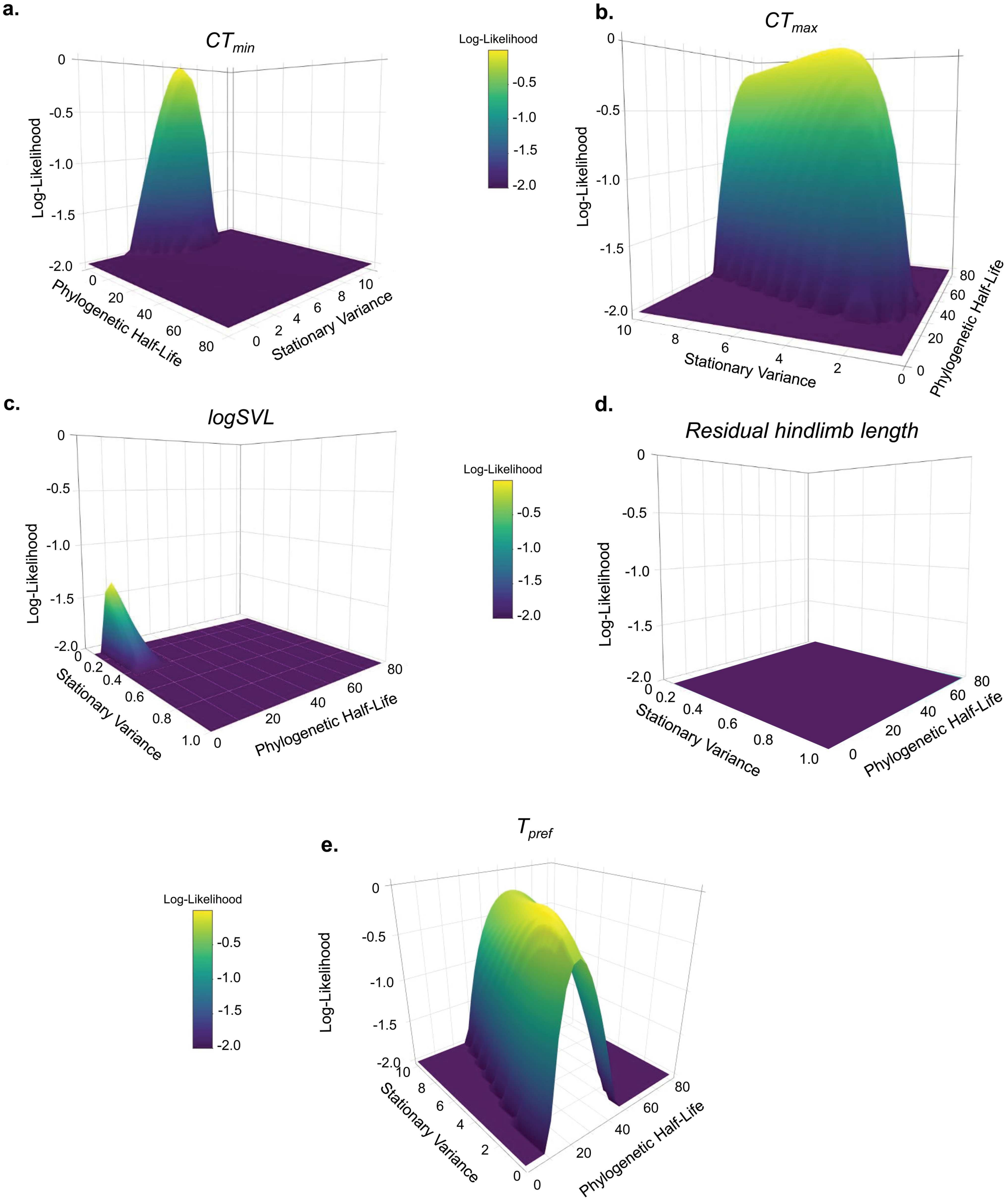

FIGURE 2. Physiological traits adapt to thermal macrohabitat, but the pace of adaptation varies among traits. Morphological traits either adapt weakly or do not adapt to the thermal environment. Likelihood support surfaces for the phylogenetic half-life ($t_{1/2}$) and the stationary variance (vy) for a regression of traits on mean annual temperature. a) Critical thermal minimum: $t_{1/2} = 0.00$ million years; $v_y = 4.09$°C2; $R^2 = 0.316$. b) Critical thermal maximum: $t_{1/2} = 32.75$ million years; $v_y = 5.20$ °C2; $R^2 = 0.116$. c) Body size: $t_{1/2} = 6.62$ million years; $v_y = 0.062$ log mm^2; $R^2 = 0.105$. d) Residual hindlimb length: $t_{1/2} = 2.30$ million years; $v_y = 0.00$ log mm^2; $R^2 = 0.000$. e) Preferred temperature: $t_{1/2} = 17.86$ million years; $v_y = 3.08$°C2; $R^2 = 0.07$. The elevated area in each plot shows all points that are within 2 support units of the best estimate. Note that the axes were swapped in panel a relative to b-e to best show the shape of the surface.

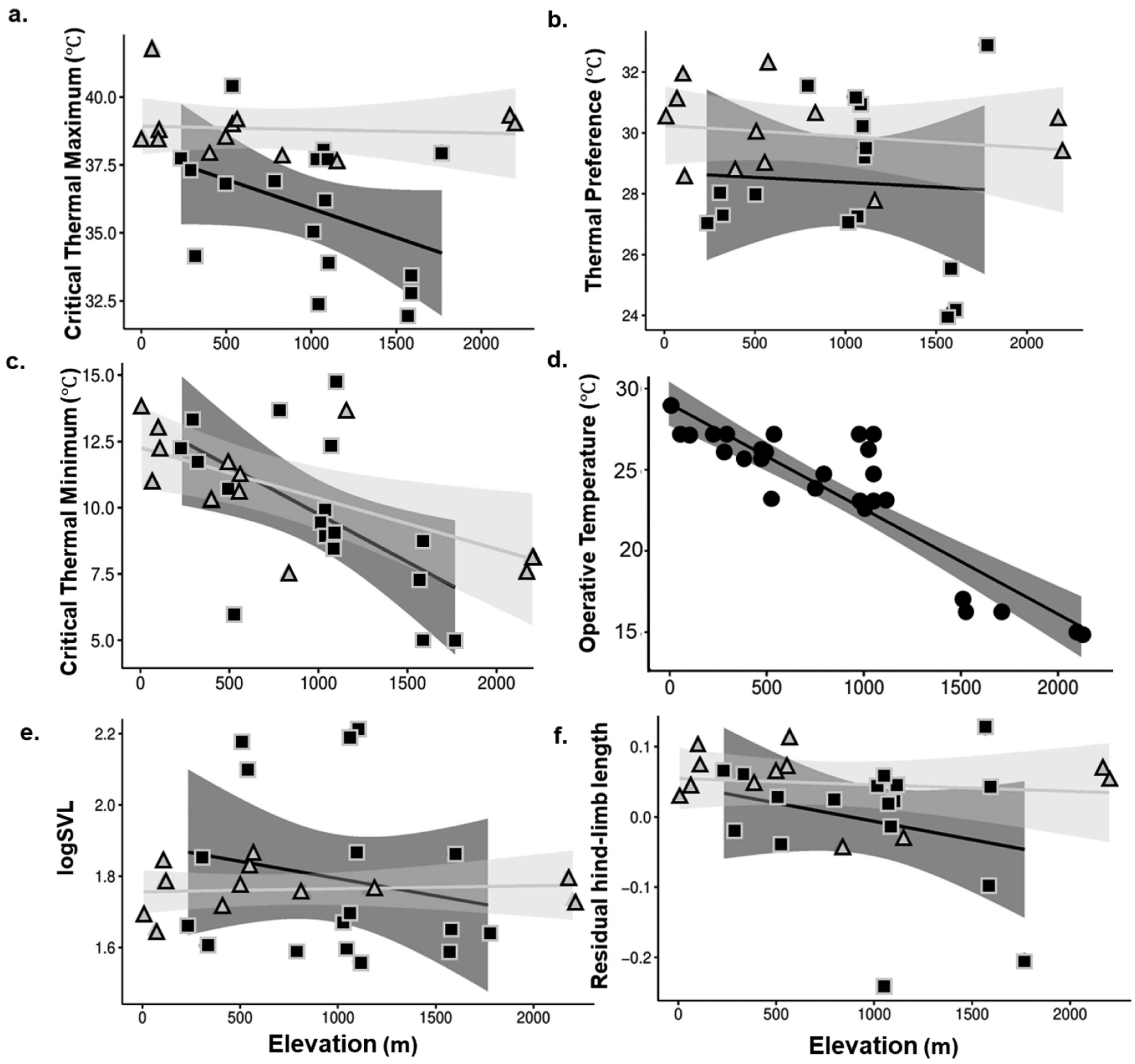

FIGURE 3. Relationships between phenotypic traits, operative temperatures, and elevation. a) The critical thermal maximum (CT_{max}) decreases with elevation in closed-canopy species but remains stable in open/edge-canopy species. phylANCOVA examining the impact of thermal microhabitat on heat tolerance: $F_{1,27} = 14.86$, $P = 0.001$. b) Thermal preference does not vary across elevation. phylANCOVA: $F_{1,26} = 3.73$, $P = 0.064$. c) For both closed- and open-canopy species, the critical thermal minimum (CT_{min}) declines with elevation. phylANCOVA: $F_{1,27} = 1.09$, $P = 0.3061$. d) The average operative temperature for each species decreases with elevation. Linear model regression: $F_{1,27} = 102$, $P = <0.0001$ adjusted $R^2 = 0.78$. (no difference between PGLS and non-phylogenetic regression). Neither canopy-use category nor elevation predicted (e) body size or (f) residual hindlimb length variation (all $P > 0.05$). Closed-canopy species are shown with black squares, and open/edge-canopy species are given in gray triangles. The shaded areas denote the 95% confidence interval.

across all structural microhabitat types. As such, we could anticipate that ecomorphological and ecophysiological diversity are likewise unaligned. Indeed, structural habitat use does not predict physiological variation (i.e., ecomorphs do not cluster in physiological trait space), and canopy use does not predict morphological specialization (i.e., open/edge and closed habitat species do not form separate clusters in morphological trait space). Physiological traits respond to a combination of canopy use (closed vs. edge/open) and thermal macroclimate (across elevation). Morphological diversity, by contrast, responds primarily to structural habitat use (as long established; reviewed in Losos 2009) and, to a lesser extent, to thermal macrohabitat across elevation.

Consistent with a 'stages' model, we found that rates of evolution vary among traits. But, morphological and physiological rates do not cluster separately. Cold tolerance evolution seems to be notably quick relative to other traits, more so than could be explained

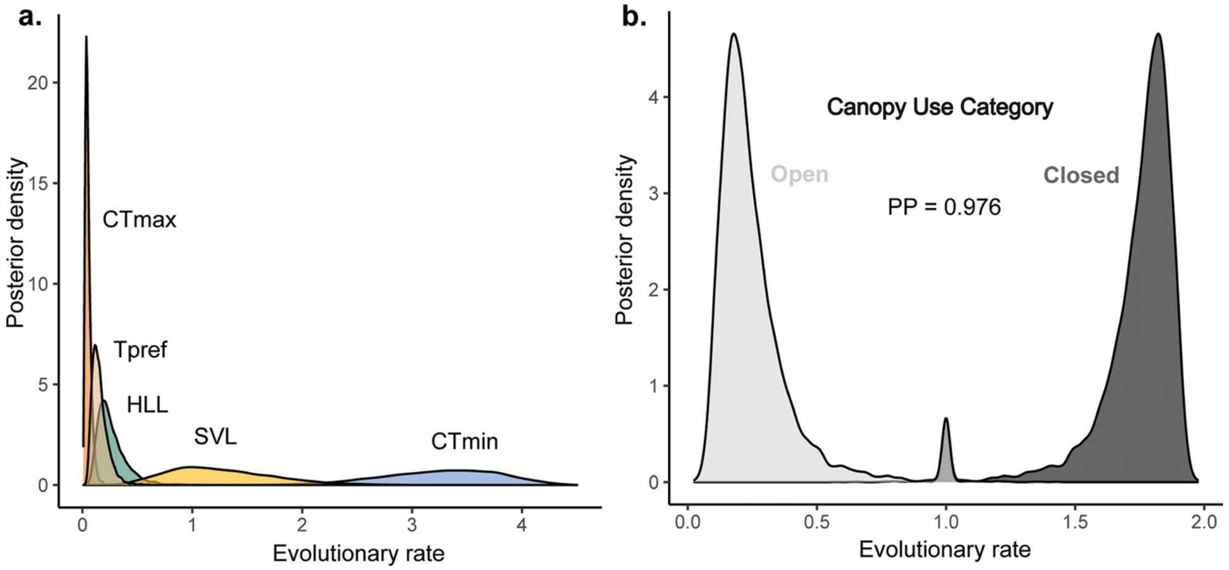

FIGURE 4. Cold tolerance evolution outpaces all other traits. Physiological traits evolve more slowly in open/edge habitat species. a) The posterior densities of the evolutionary rates for all traits of interest (red = heat tolerance (CT_{max}); blue = cold tolerance (CT_{min}); beige = preferred temperature (Tpref); green = residual hindlimb length (HLL); yellow = snout-to-vent length (SVL)). b) The posterior densities of rates of physiological trait evolution colored by canopy-use category. Physiological evolution is faster in closed-canopy species. This result is driven by CT_{max} and Tpref, as univariate models with those traits were significant (both PP > 0.95), whereas CTmin was non-significant (PP = 0.401).

simply by differences in the scale of the underlying data (**Supplementary Fig. S6**). These patterns may point to a biological explanation; however, we tentatively interpret the comparison with morphological traits since they are measured in different units. When compared to CT_{min} the slower rates of CT_{max} and T_{pref} evolution may reflect behavioral buffering through thermoregulatory behavior (i.e., the "Bogert effect"; Bogert 1949; Huey et al. 2003; Muñoz 2022). As they are diurnal, anoles are active when the environment is more thermally heterogeneous, and this fine-scale thermal variation facilitates behavioral thermoregulation (Muñoz et al. 2014a; Muñoz and Bodensteiner 2019). At night, by contrast, environments thermally compress and become progressively cooler with elevation (Ghalambor et al. 2006; Muñoz and Bodensteiner 2019). Coupled with inactivity at night, reduced behavioral buffering capacity promotes rapid cold tolerance adaptation to local thermal conditions in anoles (Muñoz et al. 2014; Salazar et al. 2019). Indeed, CT_{min} declines with elevation in all species (**Fig. 3**), rapidly evolves to match the thermal environment (**Fig. 2**), and its rate of evolution is unrelated to canopy use (**Fig. 4**). Heat tolerance and the preferred temperature, by contrast, exhibit protracted evolutionary lags in adaptation to the thermal environment (i.e., longer phylogenetic half-life) (**Fig. 2**). This disparity in behavioral buffering may have far-reaching signatures on physiological evolution: a faster rate of cold tolerance evolution relative to heat tolerance evolution is observed across a wide range of vertebrate and invertebrate lineages (e.g., Qu and Wiens 2020; Bennett et al. 2021; Bodensteiner et al. 2021).

Physiological evolution responds to canopy use, as well as thermal habitat: heat tolerance decreases with elevation in species that occupy closed-canopy habitats but remains stable in species that occupy open/edge habitats. Closed-canopy habitats tend to be cool and thermally stable (i.e., limited spatiotemporal thermal heterogeneity), which increases the costs of thermoregulation by amplifying transit distances between thermally optimal perches (Huey 1974; Sears et al. 2016). Edge/open habitats, by contrast, afford greater microscale thermal heterogeneity in sun and shade patches, which facilitates behavioral thermoregulation (Hertz 1992; Sears et al. 2016). Consistent with steeper costs of thermoregulation, the Bogert effect is weaker, and rates of heat tolerance and preferred temperature evolution are faster in closed-canopy species than in their open/edge habitat counterparts (**Fig. 4**).

Although morphological and physiological evolution is largely unaligned, we detected a weak inverse relationship between thermal macrohabitat and body size, such that high-elevation species are smaller than those found near sea level. Inverse Bergmann's clines (i.e., smaller body sizes in cooler environments) are common in squamates (Ashton and Feldman 2003; Muñoz et al. 2014b). Smaller lizards warm and cool more rapidly (Bogert 1949; Stevenson 1985; Penniket and Cree 2015), which may allow for more precise thermoregulation in cold montane environments, and potentially enhance hours of activity during the day (Ashton and Feldman 2003). Another (non-mutually exclusive) possibility is that temperature is a surrogate for more proximate predictors of body size evolution, like primary productivity and insect abundances (Hodkinson 2005; Del Grosso et al. 2008). We do note that the relationship between body size and elevation was not particularly strong. Nonetheless, given that body size is a primary feature of ecomorph identity (Williams 1972; Losos 2009; Mahler et al. 2010), ecomorphology and ecophysiology are at least partially intertwined.

Putting these pieces together, morphological and physiological evolution (and their corresponding selective pressures) can largely be isolated, setting up the possibility for "early" and "late" bursts of evolution expected under a stages model of adaptive radiation. Following Williams' (1972) model, we would expect "early bursts" for morphological evolution and "late bursts" for physiological evolution. Yet, contrary to expectation, we found no compelling evidence for distinct pulses of diversification consistent with a "stages" model of adaptive radiation. We may not detect have detected an 'early burst' of body size evolution because this trait responds to both structural habitat use and thermal environment (**Supplementary Fig. S7**; **Supplementary Table S12**). More broadly, that body size responds to numerous selective pressures may help explain why "early bursts" of body size evolution are exceedingly rare across numerous animal adaptive radiations (Harmon et al. 2010).

In contrast to the other phenotypic traits, cold tolerance evolution is exceptional on several fronts. Cold tolerance evolves rapidly and independently from the other physiological traits. These exceptional patterns of cold tolerance evolution point to an adaptive radiation driven, at least in part, by ecophysiological specialization across elevation. Notably, cold tolerance does not vary according to canopy use, so co-occurring anoles do not partition habitat based on this feature: instead, close relatives diverge across elevation, distinguished by their ability to withstand cold temperatures. Hispaniola has been subject to climatic fluctuations during its history, including relatively warm periods that may have facilitated upslope movements of anoles (e.g., Crausbay et al. 2015; Muñoz et al. 2022): as the climate cooled following such periods, high-elevation lizards may have rapidly adapted their cold tolerances to prevailing thermal conditions.

The Surprising Case of Not-so-parallel Evolution in Caribbean Anoles

Once ecomorphs arise, they often proliferate into species-rich radiations, producing upwards of a dozen morphologically similar species (Losos 2009; Muñoz et al. 2023). Starting with classic work by Williams (1972) a half-century ago, adaptive radiation in anoles has been proposed to occur in stages, with initial divergence occurring in body size and limb proportions and subsequent divergence involving physiological specialization (Hertz et al. 2013). On Puerto Rico (Rand 1964; Williams 1983), within-ecomorph niche partitioning certainly occurs along the sun-shade axis, prompting specialization in heat tolerance to "warm" and "cool" microclimatic niches (Gunderson et al. 2018). Although physiological data are not available for Cuban anoles, within-ecomorph lability in canopy use follows a similar pattern, suggesting that comparable processes are at play (Ruibal 1961; Schettino et al. 2010). Notably, early notions about the "stages" hypothesis in anoles were developed from observations on Puerto Rico.

Simply put, parallel patterns are not borne out on Hispaniola. Instead, within-ecomorph speciation appears to largely involve allopatric isolation across biogeographic boundaries and parapatric isolation across elevation (e.g., Glor et al. 2003; Wollenberg et al. 2013; Geneva et al. 2015; MacGuigan et al. 2017; Ng et al. 2017), without concomitant divergence in physiology (except for cold tolerance specialization across elevation). For example, the trunk-crown species *Anolis coelestinus* and *A. chlorocyanus* are morphologically and physiologically indistinguishable, but are separated by Merten's line, the biogeographic boundary between Hispaniola's northern and southern paleo-islands (Iturralde-Vinent and MacPhee 1999; Glor and Warren 2011). The trunk anoles (the "distichoids") are physiologically and morphologically quite similar; breaks in gene flow within this lineage appear to be accompanied by dewlap color shifts to aid in species recognition, rather than physiological shifts (Ng and Glor 2011; Ng et al. 2011; Lambert et al. 2013). Likewise, the morphologically similar trunk-ground anoles (the "cybotoids") are indistinguishable in heat tolerance, but vary in cold tolerance depending on elevation, with some species separated across elevation according to forest type (Muñoz et al. 2014a, 2022). When in sympatry, close relatives tend to diverge in fine-scale structural niche use. For example, when *A. cybotes* and *A. strahmi* (2 trunk-ground species) co-occur, the former is more often observed on tree trunks while the latter is more often observed on boulders/cliff faces (Schwartz 1989; M. Muñoz, pers. obs.), with subtle specialization in body size and hindlimb length to accommodate these shifts in structural habitat use (Glor et al. 2003). Such within-ecomorph specialization in structural niche use and morphological specialization may also help explain why morphology does not exhibit a classic signature of "early burst" divergence, instead evolving progressively through the radiation's history.

Stark differences in ecophysiological evolution among islands is perhaps surprising given convergent ecomorphological evolution across the Greater Antilles (Losos et al. 1998; Mahler et al. 2013), and that convergence in stages has been observed in cichlid fishes, another classic case of replicated adaptive radiation (Ronco et al. 2021). Perhaps independent replays of the evolutionary "tape" across islands resulted in repeatable morphological evolution in anoles because lowland forests across the Greater Antilles are structurally similar, providing a shared adaptive substrate among islands (Losos 2009). The thermal habitat structure, in contrast, varies dramatically across islands (i.e., in topography, canopy structure, total high-elevation area, and maximum elevation). Therefore, the idiosyncrasies of physiological evolution might correspondingly reflect numerous historical contingencies across islands.

Hispaniola bears > 3,000 km^2 of high-elevation habitat, which has been an engine for anole diversity, particularly cloud forest endemics (Frishkoff et al. 2022) that are poor thermoregulators and relatively cool-adapted. In fact, there is no "closed-canopy" trunk-ground anole on Hispaniola may be because this thermal niche is occupied by generalist cloud forest species.

Concluding Thoughts on the Generalizability of the "stages" Hypothesis in Adaptive Radiation

Despite a compelling theoretical basis and numerous favorable ingredients, empirical support for evolution via independent stages in Hispaniola anoles is equivocal. Just like there is not a single definition of adaptive radiation (Gillespie et al. 2020), there is not a single set of processes or patterns associated with this phenomenon, even within anoles, a system so often characterized by replicated evolution (Losos 2009; Mahler et al. 2013; Muñoz et al. 2023). To be clear, we do not advocate metaphorically throwing the baby out with the bathwater. The "stages" hypothesis intuitively captures the multidimensional nature of adaptive radiation and provides testable hypotheses for the evolution of such diversity, and it is consistent with evolutionary patterns observed on other Caribbean islands. But many proposed theoretical features of adaptive radiation result in patchwork empirical outcomes. For example, despite strong theoretical support, few empirical studies find evidence for "early burst" evolution in body size during adaptive radiation (Harmon et al. 2010). This is not to say that 'early burst' evolution is an unreasonable expectation of adaptive radiation. Instead, this empirical outcome suggests that body size per se does not tend to saturate early in a lineage's history, and perhaps evolves in response to numerous ecological/environmental pressure,s that arise across a lineage's history. Much emphasis has been placed on shared "stages" of evolution during adaptive radiation (Streelman and Danley 2003; Gavrilets and Losos 2009), but the reality is likely to be much more nuanced. Our results encourage a deeper consideration of whether and why "general" features of adaptive radiation, like independent stages, should be met and, perhaps more informatively, whether and why they should be expected to fall apart (Bolnick et al. 2018).

SUPPLEMENTARY MATERIAL

Data available from the Dryad Digital Repository: https://dx.doi.org/10.5061/dryad.8pk0p2ntg.

FUNDING

This research was supported by funding awarded to M.M.M. from the National Science Foundation DEB-2054569 and funding awarded to B.B. from Yale Insititute for Biospheric Studies Small Grant Program and MacMillan International Dissertation Research Fellowship.

ACKNOWLEDGMENTS

We thank Marcos Rodríguez, Cristian Marte, Miguel Landestoy, Ximena Baquero Gonzalez, Bianka M. Sanó Pérez, Reveca Ramirez, Jesus Vega, and the Muñoz lab members for assistance in the field. We additionally would like to thank D.L. Mahler who provided helpful information on the natural history of several Hispaniolan anole species.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

All research was done in accordance with IACUC approved protocols and with permission from all necessary agencies: Ministerio de Medio Ambiente y Recursos Naturales Autorización No. 48875679.

REFERENCES

Ackerly D., Schwilk D., Webb C. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. Ecology. 87(7):S50–S61.

Algar A.C., Mahler D.L., Glor R.E., Losos J.B., Losos J.B. 2013. Niche incumbency, dispersal limitation and climate shape geographical distributions in a species-rich island adaptive radiation. Glob. Ecol. Biogeogr. 22(4):391–402.

Allen J.A. 1877. The influence of physical conditions in the genesis of species. Radical review. 1:108–140.

Alomar N., Bodensteiner B.L., Hernández-Rodríguez I., Landestoy M.A., Domínguez-Guerrero S.F., Muñoz M.M. 2024. Comparison of hydric and thermal physiology in an environmentally diverse clade of Caribbean anoles. Integr. Comp. Biol.: 1–13.

Armbruster W.S., Pélabon C., Bolstad G.H., Hansen T.F. 2014. Integrated phenotypes: understanding trait covariation in plants and animals. Philos. Trans. R. Soc. London, Ser. B. 369(1649):20130245.

Ashton K.G., Feldman C.R. 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. Evolution. 57(5):1151–1163.

Bakken G.S. 1992. Measurement and application of operative and standard operative temperatures in ecology. Am. Zool. 32(2):194–216.

Beaulieu J.M., Jhwueng D.C., Boettiger C., O'Meara B.C. 2012. Modeling stabilizing selection: expanding the Ornstein–Uhlenbeck model of adaptive evolution. Evolution. 66(8):2369–2383.

Beaulieu J.M., and O'Meara B.C. 2015. OUwie: analysis of evolutionary rates in an OU framework. R package version 1 1: 1-9. http://cran.r-project.org/web/packages/OUwie/index.html

Bennett J.M., Sunday J., Calosi P., Villalobos F., Martínez B., Molina-Venegas R., Araújo M.B., Algar A.C., Clusella-Trullas S., Hawkins B.A., Keith S.A., Kühn I., Rahbek C., Rodríguez L., Singer A., Morales-Castilla I., Olalla-Tárraga M.A. 2021. The evolution of critical thermal limits of life on Earth. Nat. Commun. 12(1):1198.

Bergman C. 1847. Ueber die Verhaltnisse der Wa¨ rmeo¨konomie des Thieres zu ihrer Gro¨sse. Gottinger Stud. 3:595–708.

Bodensteiner B.L., Agudelo-Cantero G.A., Arietta A.A., Gunderson A.R., Muñoz M.M., Refsnider J.M., Gangloff E.J. 2021. Thermal adaptation revisited: how conserved are thermal traits of reptiles and amphibians? J Exp Zool A: Ecol Integr Physiol. 335(1):173–194.

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

Bolnick D.I., Barrett R.D., Oke K.B., Rennison D.J., Stuart Y.E. 2018. (Non) parallel evolution. Annu. Rev. Ecol. Evol. Syst. 49:303–330.

Burress E.D., Martinez C.M., Wainwright P.C. 2020. Decoupled jaws promote trophic diversity in cichlid fishes. Evolution. 74(5):950–961.

Burress E.D., Muñoz M.M. 2022. Ecological opportunity from innovation, not islands, drove the anole lizard adaptive radiation. Syst. Biol. 71(1):93–104.

Crausbay S.D., Martin P.H., Kelly E.F. 2015. Tropical montane vegetation dynamics near the upper cloud belt strongly associated with a shifting ITCZ and fire. J. Ecol. 103(4):891–903.

Del Grosso S., Parton W., Stohlgren T., Zheng D., Bachelet D., Prince S., Hibbard K., Olson R. 2008. Global potential net primary production predicted from vegetation class, precipitation, and temperature. Ecology. 89(8):2117–2126.

Felsenstein J. 1988. Phylogenies and quantitative characters. Annu. Rev. Ecol. Syst. 19(1):445–471.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in adaptive radiations. PLoS Biol. 4(11):e373.

Frishkoff L.O., Lertzman-Lepofsky G., Mahler D.L. 2022. Evolutionary opportunity and the limits of community similarity in replicate radiations of island lizards. Ecology Lett. 25(11):2384–2396.

Gavrilets S., Losos J.B. 2009. Adaptive radiation: contrasting theory with data. Science. 323(5915):732–737.

Geneva A.J., Hilton J., Noll S., Glor R.E. 2015. Multilocus phylogenetic analyses of Hispaniolan and Bahamian trunk anoles (distichus species group). Mol. Phylogenet. Evol. 87:105–117.

Ghalambor C.K., Huey R.B., Martin P.R., Tewksbury J.J., Wang G. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. Integr. Comp. Biol. 46(1):5–17.

Gillespie R.G., Bennett G.M., De Meester L., Feder J.L., Fleischer R.C., Harmon L.J., Hendry A.P., Knope M.L., Mallet J., Martin C., Parent C.E., Patton A.H., Pfennig K.S., Rubinoff D., Schluter D., Seehausen O., Shaw K.L., Stacy E., Stervander M., Stroud J.T., Wagner C., Wogan G.O.U. 2020. Comparing adaptive radiations across space, time, and taxa. J. Hered. 111(1):1–20.

Givnish T.J. 2015. Adaptive radiation versus 'radiation'and 'explosive diversification': why conceptual distinctions are fundamental to understanding evolution. New Phytol. 207(2):297–303.

Givnish T.J., Millam K.C., Mast A.R., Paterson T.B., Theim T.J., Hipp A.L., Henss J.M., Smith J.F., Wood K.R., Sytsma K.J. 2009. Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). Proc. Roy. Soc. B: Biol. Sci. 276(1656):407–416.

Glor R., Kolbe J.J., Powell R., Larson A., Losos J.B. 2003. Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). Evolution. 57(10):2383–2397.

Glor R.E., Warren D. 2011. Testing ecological explanations for biogeographic boundaries. Evolution Int. J. Org Evolution. 65(3):673–683.

Gunderson A.R., Mahler D.L., Leal M. 2018. Thermal niche evolution across replicated Anolis lizard adaptive radiations. Proc. Biol. Sci. 285(1877):20172241.

Hansen T.F., Pienaar J., Orzack S.H. 2008. A comparative method for studying adaptation to a randomly evolving environment. Evolution Int. J. Org Evolution. 62(8):1965–1977.

Harmon L., Weir J., Brock C., Glor R., Challenger W., Hunt G., FitzJohn R., Pennell M., Slater G., Brown J. 2020. Package 'geiger'. R package version 2:1–74.

Harmon L.J., Losos J.B., Jonathan Davies T., Gillespie R.G., Gittleman J.L., Bryan Jennings W., Kozak K.H., McPeek M.A., Moreno-Roark F., Near T.J. 2010. Early bursts of body size and shape evolution are rare in comparative data. Evolution Int. J. Org Evolution. 64(8):2385–2396.

Henderson RW, Powell R. 2009. Natural history of West Indian reptiles and amphibians. Gainesville (FL): University Press of Florida.

Hertz P. 1992. Evaluating thermal resource partitioning. Oecologia. 90(1):127–136.

Hertz P., Arce-Hernandez A., Ramirez-Vazquez J., Tirado-Rivera W., Vazquez-Vives L. 1979. Geographical variation of heat sensitivity and water loss rates in the tropical lizard, Anolis gundlachi. Comp Biochem Physiol A. 62(4):947–953.

Hertz P.E., Arima Y., Harrison A., Huey R.B., Losos J.B., Glor R.E. 2013. Asynchronous evolution of physiology and morphology in *Anolis* lizards. Evolution. 67(7):2101–2113.

Hertz P.E., Huey R.B. 1981. Compensation for altitudinal changes in the thermal environment by some *Anolis* lizards on Hispaniola. Ecology. 62(3):515–521.

Hertz P.E., Huey R.B., Stevenson R. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. Am. Naturalist. 142(5):796–818.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25(15):1965–1978.

Hodkinson I.D. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. Biol. Rev. Camb. Philos. Soc. 80(3):489–513.

Höhna S., Landis M.J., Heath T.A., Boussau B., Lartillot N., Moore B.R., Huelsenbeck J.P., Ronquist F. 2016. RevBayes: bayesian phylogenetic inference using graphical models and an interactive model-specification language. Syst. Biol. 65(4):726–736.

Huey R.B. 1974. Behavioral thermoregulation in lizards: importance of associated costs. Science. 184(4140):1001–1003.

Huey R.B. 1982. Temperature, physiology, and the ecology of reptiles. In: Gans C., Pough F.H., editors. Biology of the Reptilia. Cambridge (MA): Academic Press. p. 25–91.

Huey R.B., Hertz P.E., Sinervo B. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. Am. Naturalist. 161(3):357–366.

Huey R.B., Webster T.P. 1976. Thermal biology of *Anolis* lizards in a complex fauna: the Christatellus group on Puerto Rico. Ecology. 57(5):985–994.

Iturralde-Vinent M., MacPhee R.D. 1999. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. Bulletin of the AMNH. 238:1–95.

James F.C. 1970. Geographic size variation in birds and its relationship to climate. Ecology 51:365–390.

Kassambara A. 2022. ggcorrplot: Visualization of a Correlation Matrix using "ggplot2." R package version 0.1.4, https://CRAN.R-project.org/package=ggcorrplot

Lambert S.M., Geneva A.J., Mahler D.L., Glor R.E. 2013. Using genomic data to revisit an early example of reproductive character displacement in Haitian *Anolis lizards*. Mol. Ecol. 22(15):3981–3995.

Lande R., Arnold S.J. 1983. The measurement of selection on correlated characters. Evolution. 37:1210–1226.

Leahy L., Scheffers B.R., Andersen A.N., Hirsch B.T., Williams S.E. 2021. Vertical niche and elevation range size in tropical ants: implications for climate resilience. Divers. Distrib. 27(3):485–496.

Lobry J.R., Bel-Venner M.C., Bogdziewicz M., Hacket-Pain A., Venner S. 2023. The CV is dead, long live the CV! Methods Ecol. Evol. 14(11):2780–2786.

Losos J.B. 1990. The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. Evolution Int. J. Org Evolution. 44(5):1189–1203.

Losos JB. 2009. Lizards in an evolutionary tree: ecology and adaptive radiation of anoles. Berkeley (CA): University of California Press.

Losos J.B., Jackman T.R., Larson A., Queiroz K.D., Rodríguez-Schettino L. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science. 279(5359):2115–2118.

Losos J.B., Leal M., Glor R.E., De Queiroz K., Hertz P.E., Rodríguez Schettino L., Chamizo Lara A., Jackman T.R., Larson A. 2003. Niche lability in the evolution of a Caribbean lizard community. Nature. 424(6948):542–545.

Lutterschmidt W.I., Hutchison V.H. 1997. The critical thermal maximum: history and critique. Can. J. Zool. 75(10):1561–1574.

MacGuigan D.J., Geneva A.J., Glor R.E. 2017. A genomic assessment of species boundaries and hybridization in a group of highly polymorphic anoles (*distichus* species complex). Ecol. Evol. 7(11):3657–3671.

Mahler D.L., Ingram T., Revell L.J., Losos J.B. 2013. Exceptional convergence on the macroevolutionary landscape in island lizard radiations. Science. 341(6143):292–295.

Mahler D.L., Lambert S.M., Geneva A.J., Ng J., Hedges S.B., Losos J.B., Glor R.E. 2016. Discovery of a giant chameleon-like lizard (Anolis) on Hispaniola and its significance to understanding replicated adaptive radiations. Am. Naturalist. 188(3):357–364.

Mahler D.L., Revell L.J., Glor R.E., Losos J.B. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. Evolution Int. J. Org Evolution 64(9):2731–2745.

Martin C.H., Richards E.J. 2019. The paradox behind the pattern of rapid adaptive radiation: how can the speciation process sustain itself through an early burst? Annu. Rev. Ecol. Evol. Syst. 50:569–593.

May M.R., Moore B.R. 2020. A Bayesian approach for inferring the impact of a discrete character on rates of continuous-character evolution in the presence of background-rate variation. Syst. Biol. 69(3):530–544.

McGlothlin J.W., Kobiela M.E., Wright H.V., Kolbe J.J., Losos J.B., Brodie ED III. 2022. Conservation and convergence of genetic architecture in the adaptive radiation of *Anolis* lizards. Am. Naturalist. 200(5):E207–E220.

McGlothlin J.W., Kobiela M.E., Wright H.V., Mahler D.L., Kolbe J.J., Losos J.B., Brodie ED III. 2018. Adaptive radiation along a deeply conserved genetic line of least resistance in Anolis lizards. Evol. Lett. 2(4):310–322.

McQueen A., Klaassen M., Tattersall G.J., Atkinson R., Jessop R., Hassell C.J., Christie M., Symonds M.R.; Victorian Wader Study Group, Australasian Wader Studies Group. 2022. Thermal adaptation best explains Bergmann's and Allen's Rules across ecologically diverse shorebirds. Nat. Commun. 13(1):4727.

Morinaga G., Wiens J.J., Moen D.S. 2023. The radiation continuum and the evolution of frog diversity. Nat. Commun. 14(1):7100.

Münkemüller T., Boucher F.C., Thuiller W., Lavergne S. 2015. Phylogenetic niche conservatism–common pitfalls and ways forward. Funct. Ecol. 29(5):627–639.

Muñoz M.M. 2019. The evolutionary dynamics of mechanically complex systems. Integr. Comp. Biol. 59(3):705–715.

Muñoz M.M. 2022. The Bogert effect, a factor in evolution. Evolution. 76:49–66.

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

Muñoz M.M., Frishkoff L.O., Pruett J., Mahler D.L. 2023. Evolution of a model system: new insights from the study of *Anolis* lizards. Annu. Rev. Ecol. Evol. Syst. 54:475–503.

Muñoz M.M., Langham G.M., Brandley M.C., Rosauer D.F., Williams S.E., Moritz C. 2016. Basking behavior predicts the evolution of heat tolerance in Australian rainforest lizards. Evolution. 70(11):2537–2549.

Muñoz M.M., Losos J.B. 2018. Thermoregulatory behavior simultaneously promotes and forestalls evolution in a tropical lizard. Am. Naturalist. 191(1):E15–E26.

Muñoz M.M., Stimola M.A., Algar A.C., Conover A., Rodriguez A.J., Landestoy M.A., Bakken G.S., Losos J.B. 2014a. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. Proc. Biol. Sci. 281(1778):20132433.

Muñoz M.M., Wegener J.E., Algar A.C. 2014b. Untangling intra-and interspecific effects on body size clines reveals divergent processes structuring convergent patterns in *Anolis* lizards. Am. Naturalist. 184(5):636–646.

Ng J., Geneva A.J., Noll S., Glor R.E. 2017. Signals and speciation: *Anolis* dewlap color as a reproductive barrier. J. Herpetol. 51(3):437–447.

Ng J., Glor R.E. 2011. Genetic differentiation among populations of a Hispaniolan trunk anole that exhibit geographical variation in dewlap colour. Mol. Ecol. 20(20):4302–4317.

Orme D., Freckleton R., Thomas G., Petzoldt T., Fritz S., Isaac N. 2013. The caper package: comparative analysis of phylogenetics and evolution in R. R package version. 5(2):1–36.

Penniket S., Cree A. 2015. Adherence to Bergmann's rule by lizards may depend on thermoregulatory mode: support from a nocturnal gecko. Oecologia. 178(2):427–440.

Pianka E.R. 1986. Ecology and natural history of desert lizards: analyses of the ecological niche and community structure. Princeton, NJ, USA: Princeton University Press.

Qu Y.F., Wiens J.J. 2020. Higher temperatures lower rates of physiological and niche evolution. Proc. Biol. Sci. 287(1931):20200823.

R Core Team. 2021. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rand A.S. 1964. Ecological distribution in anoline lizards of Puerto Rico. Ecology. 45(4):745–752.

Revell L.J. 2010. Phylogenetic signal and linear regression on species data. Methods Ecol. Evol. 1(4):319–329.

Revell L.J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3(2):217–223.

Ronco F., Matschiner M., Böhne A., Boila A., Büscher H.H., El Taher A., Indermaur A., Malinsky M., Ricci V., Kahmen A., Jentoft S., Salzburger W. 2021. Drivers and dynamics of a massive adaptive radiation in cichlid fishes. Nature. 589(7840):76–81.

Ruibal R. 1961. Thermal relations of five species of tropical lizards. Evolution. 15(1):98–111.

Salazar J.C., del Rosario Castañeda M., Londono 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.

Schettino L.R., Losos J.B., Hertz P.E., de Queiroz K., Chamizo A.R., Leal M., González V.R. 2010. The anoles of Soroa: aspects of their ecological relationships. Breviora 520(1):1–22.

Schluter D. 2000. The ecology of adaptive radiation. Oxford, UK: Oxford University Press.

Schwartz A. 1989. A review of the cybotoid anoles (Reptilia: Sauria: Iguanidae) from Hispaniola. Milwaukee (WI): Milwaukee Public Museum.

Schwartz A., Henderson R.W. 1991. Amphibians and reptiles of the West Indies: descriptions, distributions, and natural history. Gainesville (FL): University Press of Florida.

Sears M.W., Angilletta M.J. Jr, Schuler M.S., Borchert J., Dilliplane K.F., Stegman M., Rusch T.W., Mitchell W.A. 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. Proc. Natl. Acad. Sci. USA. 113(38):10595–10600.

Simpson G.G., editor. 1953. The major features of evolution. New York: Columbia University Press.

Spellerberg I.F. 1972. Temperature tolerances of southeast Australian reptiles examined in relation to reptile thermoregulatory behaviour and distribution. Oecologia. 9:23–46.

Stevenson R. 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. Am. Naturalist. 125(1):102–117.

Streelman J.T., Danley P.D. 2003. The stages of vertebrate evolutionary radiation. Trends Ecol Evol. 18(3):126–131.

Stroud J.T., Moore M.P., Langerhans R.B., Losos J.B. 2024. Fluctuating selection maintains distinct species phenotypes in an ecological community in the wild. Proc. Natl. Acad. Sci. USA. 120(42):e2222071120.

Sunday J., Bennett J.M., Calosi P., Clusella-Trullas S., Gravel S., Hargreaves A.L., Leiva F.P., Verberk W.C., Olalla-Tárraga M.A., Morales-Castilla I. 2019. Thermal tolerance patterns across latitude and elevation. Philos Trans R Soc B. 374(1778):20190036.

Taylor E.N., Diele-Viegas L.M., Gangloff E.J., Hall J.M., Halpern B., Massey M.D., Rödder D., Rollinson N., Spears S., Sun B.J., Telemeco R.S. 2021. The thermal ecology and physiology of reptiles and amphibians: a user's guide. J Exp Zool A: Ecol Integr Physiol. 335(1):13–44.

Wagner G., Altenberg L. 1996. Perspective: complex adaptations and the evolution of evolvability. Evolution. 50:967–976.

Walker J.A. 2007. A general model of functional constraints on phenotypic evolution. Am. Naturalist. 170(5):681–689.

Williams EE. 1972. The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. In Dobzhansky T., Hecht M.K., Steere W.C., editors. Evolutionary biology. New York (NY): Springer. p. 47–89.

Williams EE. 1983. Ecomorphs, faunas, island size, and diverse end points in island radiations of *Anolis*. In: Huey R.B., Pianka E.R., Schoener T.W., editors. Lizard Ecology: Studies of a Model Organism. Cambridge, MA: Harvard University Press. p. 326–370.

Wollenberg K.C., Wang I.J., Glor R.E., Losos J.B. 2013. Determinism in the diversification of Hispaniolan trunk-ground anoles (*Anolis cybotes* species complex). Evolution. 67(11):3175–3190.