A multidimensional perspective on the role of behavior in evolution

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A multidimensional perspective on the role of behavior in evolution

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A multidimensional perspective on the role of behavior in evolution.

Abstract

Behavior determines how organisms interact with their environment, and has long been posited as a pacemaker for evolution. The classical view is that novel behaviors expose organisms to new selective pressures, in turn “driving” evolution. Behavior can also restrain evolutionary change. Some behaviors, such as thermoregulation, help organisms maintain a constant selective environment, thus “inhibiting” evolution. This thesis seeks to understand the role of behavior in influencing the evolutionary process.

In the first part, I test the hypothesis that the same behavior can simultaneously impede and impel evolution in different traits. I focus on the lizard, *Anolis cybotes*, from the Caribbean island of Hispaniola. Through a replicated field experiment I show that behavioral flexibility allows these lizards to maintain a constant body temperature in markedly different thermal habitats. I determine that this similarity in body temperatures is associated with physiological stasis, as the preferred temperature and heat tolerance are nearly identical among populations. I demonstrate that the behavioral change allowing lizards to maintain a constant body temperature involves a perch switch. Finally, I demonstrate that this shift in structural habitat use from trees at low elevation to rocks at high elevation in turn impels morphological evolution in traits associated with rock use, and that these traits are likely genetically based. Thus, a perch switch to rocks at high elevation is simultaneously impeding physiological evolution, whilst impelling morphological evolution.

In the second part of my study, I asked whether rates of evolution differ among physiological traits, and how thermoregulation influences these rates. I found that cold tolerance evolves significantly faster than heat tolerance in the cybotoid anoles, a clade of anoles that contains *A. cybotes* and its relatives. I demonstrate that thermal variation is considerably greater during the day than at night and, at high elevation, nighttime temperatures are so cold that they would incapacitate most lizards. In the absence of thermal refuges and behavioral buffering, lizards at high elevation have no choice but to adapt their physiology. Thus, the ability to thermoregulate during, but not at night, likely influences differences in rates of evolution between heat and cold tolerance.

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Para mi querida Mamita.

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The cradle rocks above an abyss, and common sense tells us that our existence is but a brief crack of light between two eternities of darkness.
–Vladimir Nabokov (Speak, Memory)

Six years came and went in the blink of an eye. And in what feels now like the briefest beam between the slats of a cradle, I learned and laughed and loved and lost. The future, too, is full of light and promise, but it will be a different place, a different time, and a different flash. There were many who shared this moment with me, and I know that I cannot thank everyone, but I will try.

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Introduction

Behavior determines how organisms interact with their environments and can greatly influence the magnitude and direction of evolution. Through behavior organisms colonize new environments and exploit novel resources, in turn impelling evolution (Mayr 1959, 1963). Behavior can also shield organisms from variation in the environment, thus inhibiting evolutionary change (Bogert 1949). However, despite the power of behavior to enhance or erode selection, there is surprisingly little known about how behavior influences the evolutionary process. The goal of this thesis is to expand our understanding of the role of behavior in evolution.

Some of the earliest known work on the role of behavior in evolution came from the field of reptilian thermal biology. Up through the early twentieth century, it was generally thought that supposed 'cold-blooded' animals, such as reptiles, were consummate thermoconformers, essentially at the whim and mercy of the environment. This view changed drastically with the publication of a monograph in 1944 by Raymond Cowles and Charles Bogert, in which they documented that the body temperatures of desert reptiles often varied considerably from the temperature of the environment, and suggested the lizards actively control their core temperature through behavioral choices in basking and retreat spots. Based on these observations, Bogert (1949) proposed that behavioral thermoregulation inhibits physiological evolution by shielding organisms from sub-optimal variation in the environment, and suggested that such behavioral inhibition was a pervasive factor in evolution.

The next decade saw the advent of a contrasting view on the role of behavior in evolution. Based on decades spent observing the ecological diversity of tropical birds, Ernst Mayr (1959, 1963) believed that the origin of such staggering disparity lay in behavior. Almost universally, Mayr (1963) claimed, behavior initiates shifts into novel environments or to the use of novel resources. The behavioral shifts, in turn, foment the evolution of novel adaptations.

Mayr and Bogert came to opposite conclusions on the role of behavior in evolution. The subsequent decades saw a flurry of work focusing on linking behavioral shifts to evolution, or to the lack thereof. Behavioral drive has been linked with prolific evolutionary diversification in larger-brained birds, leading to greater diversity in body size (Sol and Price 2008) and greater species richness (Nikolakakis et al. 2003; Sol et al. 2005). Behavioral inhibition, meanwhile, has been implicated in the evolutionary retention of physiological tolerances exhibited by ectothermic organisms (Huey 1982; Huey et al. 2003).

However, both the 'behavioral drive' and 'behavioral inertia' hypotheses are limited because they focus on a single possible evolutionary outcome for an organism's behavior. A species' niche is multidimensional, defined by the abiotic and biotic factors that determine where a species can persist. As Lewontin (1983) argued, behavior is the key trait that links species' niche dimensions, and may simultaneously influence selection in opposing directions for different traits. Specifically, Levins and Lewontin (1985) posited that a single behavior, such as perch use, simultaneously influences multiple aspects of a species' ecology, such as predation, thermoregulation, and predator evasion. These interactions aong niche dimensions lead to different selective pressures on different traits. Huey et al. (2003) expanded on this idea by specifically linking behavioral drive and behavioral inertia to tradeoffs in niche evolution. Stasis along one niche dimension, they argued, is achieved by shifts in resource use that precipitate evolution along another.

Through my dissertation my goal was to bridge this conceptual gap between unidimensional ideas (behavioral drive, behavioral inertia) and the multidimensional niche. For my first chapter (Chapter 1), I empirically tested Levin's and Lewontin's (1985) hypothesis that evolutionary stasis along one dimension is achieved through divergence along another. I found that perching behavior has different evolutionary consequences for physiological and morphological traits in the tropical lizard, *Anolis cybotes*.

For the next chapter (Chapter 2), I provide a conceptual background for the Levins-Lewontin Effect. I used my empirical work on *Anolis cybotes* from Chapter 1 to affirm their hypothesis. I then discussed the relevance of the Levins-Lewontin Effect to the concept of niche conservatism (NC). Through a literature search I demonstrated that, although it may be occurring, most NC studies are unidimensional and, therefore, cannot examine the Levins-Lewontin Effect. Further, I found that most multidimensional studies do find evidence for both stasis and divergence in different niche traits, but still lack an understanding of how behavioral shifts are implicated in resource use.

In the next chapter (Chapter 3), I considered whether limitations on thermoregulatory behavior influence rates of trait evolution. Specifically, because thermal heterogeneity is much greater during the day than during the cold night, thermoregulation should be more effective at shielding organisms from selection on upper, rather than lower tolerances. Consistent with my hypothesis, I found that cold tolerance evolves considerably faster than heat tolerance in a clade of tropical lizards. I demonstrated that lizards do not have warm thermal refuges at high elevation and, in the absence of behavior, must physiologically adapt.

In my next chapter (Chapter 4), I examine evolution in one of the morphological correlates of behavioral thermoregulation – body size. Using a model that can parse the relative effects of intra- and interspecific effects on body size clines, I found that lizards on Hispaniola and Cuba both exhibit inverse Bergmann's clines – lizards are smaller at high elevation – but that these clines evolved through distinct mechanisms. On Cuba, intraspecific effects (local adaptation and phenotypic plasticity) lead to the inverse size cline, whereas interspecific effects (size-ordered species turnover) produced a similar cline in Hispaniola.

Finally, the last chapter (Chapter 5) departs from the topic of behavior to examine ecological speciation in a phenotypically diverse species of lizard, *Anolis marmoratus*, from Guadeloupe. I found that differences in lizard head coloration (blue in *A. m. speciosus*; yellow in *A. m. inornatus*) are consistent with different selective pressures in the different habitats where each subspecies is found. Using genetic analysis, I determined that differences in coloration evolved in the face of high gene flow. These results suggest that *A. marmoratus* represents a stable endpoint between selection and gene flow, and an early stage along the ecological speciation continuum.

Overall, my work suggests that the role of behavior is more complex than previously thought. Interactions among niche dimensions lead niche stasis and lability to simultaneously occur. A better understanding of the role of behavior in evolution demands a multidimensional perspective.

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Chapter 1

Behavioral inertia meets behavioral drive: behavioral shifts induce physiological stasis and morphological divergence

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One Sentence Summary: Behavior impedes physiological evolution whilst impelling morphological in high elevation populations of *Anolis cybotes*.

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Abstract

By serving as a conduit for organism-environment interactions, behavior has the potential to avoid or enhance natural selection. Identification of the precise link between behavior, resource use, and evolution along multiple niche dimensions is required to fully understand the role of behavior in evolution. Through replicate field and laboratory analyses, we show that thermoregulatory behavior precludes physiological evolution in montane populations of the tropical lizard, *Anolis cybotes*. Thermoregulation is accomplished through a switch from trees at low elevation to rocks at high elevation. We demonstrate that this behavioral shift in resource use is associated with the evolution of flatter heads and shorter hindlimbs in high elevation populations. Thus, phenotypic evolution is the result of interactions among niche dimensions that are mediated by organismal behavior.

Main Text

Variation in organismal behavior has a profound impact on the pace and magnitude of evolution in the wild (Huey et al. 2003; Duckworth 2009). Ernst Mayr (1959, 1963) argued that behavioral shifts in resource use precipitate evolution by exposing organisms to novel selective pressures, an important first step in ecological speciation (Nosil 2012), as well as rapid phenotypic diversification through adaptive radiation (Schluter 2000; Sol et al. 2005). Behavior is also known to retard evolution, a phenomenon known as the Bogert Effect (Huey et al. 2003). Charles Bogert (1949) proposed that, by buffering organisms from novel selective pressures, behaviors might preclude or reduce evolutionary change.

Thus far, examination of Mayr's and Bogert's hypotheses has involved determining whether behaviors limit or enhance diversification (discussed in Plotkin 1988; West-Eberhard 1989; Gittleman et al. 1996), largely treating these effects as independent. However, theoretical evidence suggests that behavioral drive and behavioral inertia are intrinsically linked. Levins and Lewontin (1985) proposed that behavioral shifts in resource use simultaneously induce different selective pressures in different traits. Huey et al. (2003) later expanded on this hypothesis by explicitly linking it to behavioral drive and behavioral inertia, arguing that behavioral shifts simultaneously induce different evolutionary patterns for different traits – specifically, behavioral shifts that maintain stasis along one niche dimension should induce divergence along another. Despite the fact that nearly decades have passed since the proposal by Levins and Lewontin (1985), the interactions between behavioral inertia and drive remain unresolved.

Here we examine the evolutionary consequences of behavioral shifts in resource use along different niche axes in *Anolis cybotes*, a tropical lizard that ranges from sea level to over 3,000 meters on the Caribbean island of Hispaniola. This broad elevational range corresponds with dramatic differences in thermal environments among populations of *A. cybotes* (Hertz and Huey 1981, Muñoz et al. 2014; Supp. Table 1_1). At high elevations, operative temperatures (T_e; the temperatures that non-thermoregulating lizards would attain) hovered near freezing in the mornings and were on average 11°C cooler than at low elevation (Table 1_1; Fig. 1_1). In the absence of behavioral thermoregulation, thermoconforming lizards would exhibit daily body temperature (T_b) ranges of up to 50°C at high elevations and would also exhibit mean body temperature more than 10°C cooler than lizards found near sea level. *Anolis cybotes*, however, is not a thermoconformer; rather, it uses thermoregulatory behavior to adjust its body temperature, basking in the sun to warm up and moving to shade to cool down. Thermoregulation is remarkably effective; mean body temperatures varied by little more than 1°C among populations, hovering between 29°C and 31°C for all localities, and high elevation lizards experienced only a quarter of the T_b variation through the course of the day as they would have had they been thermoconformers (Fig. 1_1; Table 1_1).

Consistent with the 'Bogert Effect', the similarity in body temperatures among populations is associated with stasis in underlying thermal physiology: both preferred temperature (ANOVA: $F_{3,76} = 1.9$, $p = 0.140$) and heat tolerance (ANOVA: $F_{3,64} = 1.1$, $p = 0.349$) are nearly identical among populations (Fig. 1_1). Our results are consistent with previous studies that have found physiological stasis through behavioral thermoregulation (Hertz et al. 1983; Crowley 1985; Gunderson & Leal 2012). However, these studies have

Table 1_1. Operative temperatures (T_e) for each locality and corresponding lizard body temepratures (T_b) are given. Thermal variables are given in °C, altitude in meters, and mean T_e and T_b are given +/- 1sem. Transect refers to localities in the eastern (Cordillera Central) and western (Sierra de Baoruco) mountain chains.

Site	Transect	Altitude	T_e range	T_b range	T_e	T_b
Caamaño	East	43	20.9 – 50.9	22.9 – 36.4	30.4 ± 0.1	30.3 ± 0.4
Valle Nuevo	East	2450	3.4 – 53.4	20.1 – 33.9	27.0 ± 0.2	29.7 ± 0.3
Los Patos	West	45	22.9 – 60.9	23.7 – 34.4	32.2 ± 0.1	29.7 ± 0.3
Loma de Toro	West	2320	4.4 – 46.4	21.6 – 34.3	19.1 ± 0.1	28.9 ± 0.3

not considered the extent to which thermoregulatory behaviors require shifts in other aspects of the niche, in turn potentially impinging upon other selective pressures (Lewontin 1983; Levins and Lewontin 1985; Huey et al. 2003).

In light of Levin's and Lewontin's (1985) hypothesis, we investigated whether stasis in physiology is achieved through behavioral shifts that impel evolution in different traits. Specifically, lizards can alter heat load through behavioral shifts in microhabitat use (Huey and Pianka 1977; Adolph 1990; Angert et al. 2002), which may, in turn, be associated with morphological differentiation to different substrates. A large part of the adaptive radiation of *Anolis* lizards has involved divergence to adapt to using different parts of the structural habitat (i.e., the characteristics of the substrates the lizards use [reviewed in Losos 2009]). We found that thermal habitat quality differs dramatically between substrates at different elevations. Overall, operative temperatures were fairly similar between rocks and trees at low elevation (Table 1_2). Nonetheless, trees were a

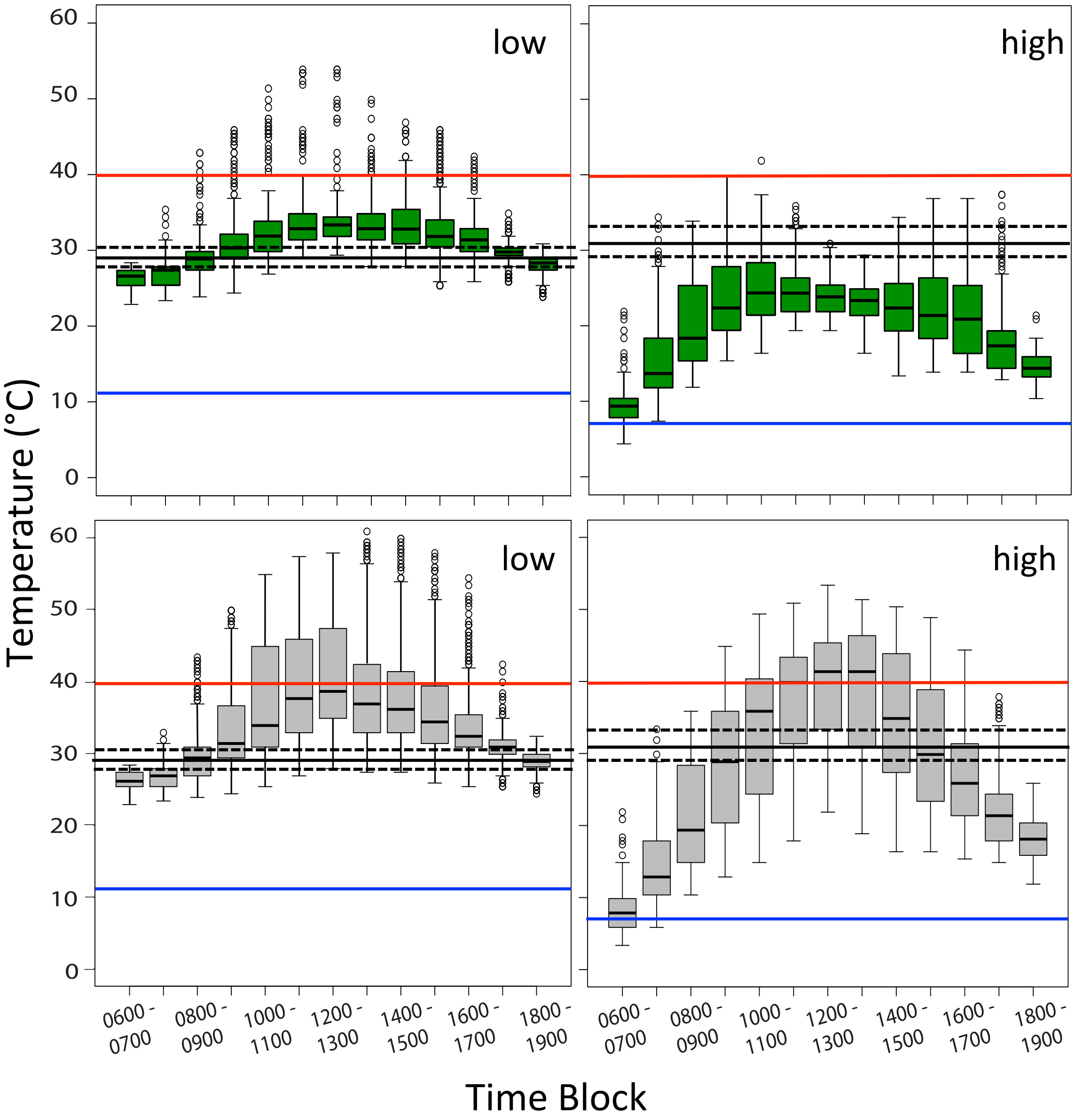

Figure 1_1. Box plots showing the variation in operative temperatures (T_e) during 1 h time blocks. Each time block summarizes temperatures collected over six consecutive days at low elevation (45 m – left panels) and high elevation (2318 m – right panels) in the Sierra de Baoruco (west). Color denotes perch type as follows: T_e on trees, green; T_e on rocks (gray). The lines show CT_{max} (red), T_p (black), T_p range (black dashed), and CT_{min} (blue) for each of the localities sampled. Results were similar for the Cordillera Central (east). Estimates of CTmin were taken from Muñoz et al. (2014).

higher quality perch than rocks (trees: d_e = 2.12, rocks: d_e = 3.62; Table 1_2) as T_e was more likely to fall within the preferred range, and less likely to exceed this range. Furthermore, low elevation lizards that perch on trees rather than rocks at low elevation also reduce the likelihood of experiencing lethal or near-lethal temperatures – T_e measured on rocks was six times more likely to exceed CT_{max} than T_e measured on trees (Fig. 1_1). At high elevation the situation was reversed. T_e measured on rocks more closely approached the preferred temperature range than on trees: in fact, nearly all T_e measurements from trees (95.6%) were lower than the preferred temperature range (Table 1_2; Supp. Table 1_2). T_e measured on rocks, in contrast, was nearly three times more often within the preferred temperature range (Fig. 1_1; Table 1_2). Thus, maintaining body temperatures within their preferred range required lizards to shift their perching behavior at high elevation. As expected by the distribution of T_e in the two environments, populations differed greatly in substrate use: lizards at low elevation were nearly always observed perching on vegetation (tree trunks and branches), whereas highland lizards were almost invariably observed basking near the ground on rocks (perch use: G = 220.0, d.f. = 1, $p < 0.001$.

We examined whether shifts in habitat use have driven adaptive shifts in morphology by examining traits that associated with the evolutionary transition to rock-dwelling in other lizards. Consistent with previous work on *A. cybotes* (Glor et al. 2003, Wollenberg et al. 2013) and other lizards (Herrel et al. 2001; Revell et al. 2007), we found that high elevation lizards possessed significantly flatter heads (ANCOVA: $p < 0.001$; Fig. 1_2; Supp. Table 1_3). Flattening can enable movements into narrow crevices and tight spaces under rocks – indeed, many high elevation *A. cybotes* quickly scramble

Table 1_2. Summary statistics evaluating thermal habitat and thermoregulation at high and low elevation. Site-specific details are given in Supp. Table 1_2. Thermal habitat quality, d_e, is given for rocks and trees and across both perch types. % below, within, and above refer to the percent of operative temperatures that fell below, within, or above the preferred temperature range, respectively. Thermoregulation was assessed through the degree of behavioral thermoregulation, d_b, and the effectiveness of thermoregulation, E, between low and high elevation sites. An asterisk denotes the perch type with the highest thermal quality at each elevation. Values closer to zero indicate a higher degree of thermoreulation (d_b) and a greater thermoregulatory efficiency (E).

Elevation	**d_e (rocks)**	**d_e (trees)**	**d_e (overall)**	**% below (rocks, trees)**	**% within (rocks, trees)**	**% above (rocks, trees)**	**d_b**	**E**
Low	3.62	2.12*	2.91	28.0, 29.3	17.3, 21.1	54.6, 49.5	1.64	0.92
High	9.12*	9.31	9.24	70.8, 95.6	9.2, 3.2	20.0, 1.2	0.75	0.44

under rocks and boulders when threatened and spend considerable amounts of time retreating under them during inclement weather (Hertz & Huey 1981; Muñoz et al. 2014). The compressed heads in highland *A. cybotes* represent a clear adaptation to a substrate shift that enabled thermoregulation.

We also found that hindlimb length shifted with substrate use, as high elevation lizard possessed significantly shorter hindlimbs than their low elevation counterparts (ANCOVA: $p < 0.001$; Fig. 1_2; Supp. Table 1_3). Some other lizards, including *A. cybotes*, have been documented to evolve shorter limbs when shifting to a rock-dwelling lifestyle (Vanhooydonck and Van Damme 1999, Arnold 1998; Glor et al. 2003), but in other cases, including some anoles, rock-dwelling species evolve longer limbs (Revell et al. 2007; Goodman et al. 2008). Hence, the association we detected may represent an adaptive shift driven by the transition from trees to rocks, but further study is needed to test the adaptive hypothesis.

To examine the possibility that the morphological changes we observed might be a plastic response induced by rock dwelling or developmental temperature rather than an evolved response, we conducted a common garden experiment in the laboratory. We collected gravid females from high and low elevation sites and reared their offspring under common conditions. We found that the same morphological differences persisted between populations – high elevation lizards have significantly flatter heads and shorter hindlimbs than low elevation lizards, supporting the hypothesis that morphological differences are genetically determined (Supp. Table 1_3).

Our results confirm that physiological stasis is achieved through a behavioral shift that concomitantly induces morphological divergence. These results suggest that a behavioral switch in perch use simultaneously affects the fitness landscape in different ways for different traits. Although behavioral drive and behavioral inertia have typically been examined unidimensionally, when considered together in a multidimensional framework, we find that they are not independent of each other. Further, it is likely that the interaction between drive and inertia is even more complex than demonstrated here. Levins and Lewontin (1985) proposed that even a single interaction between traits likely

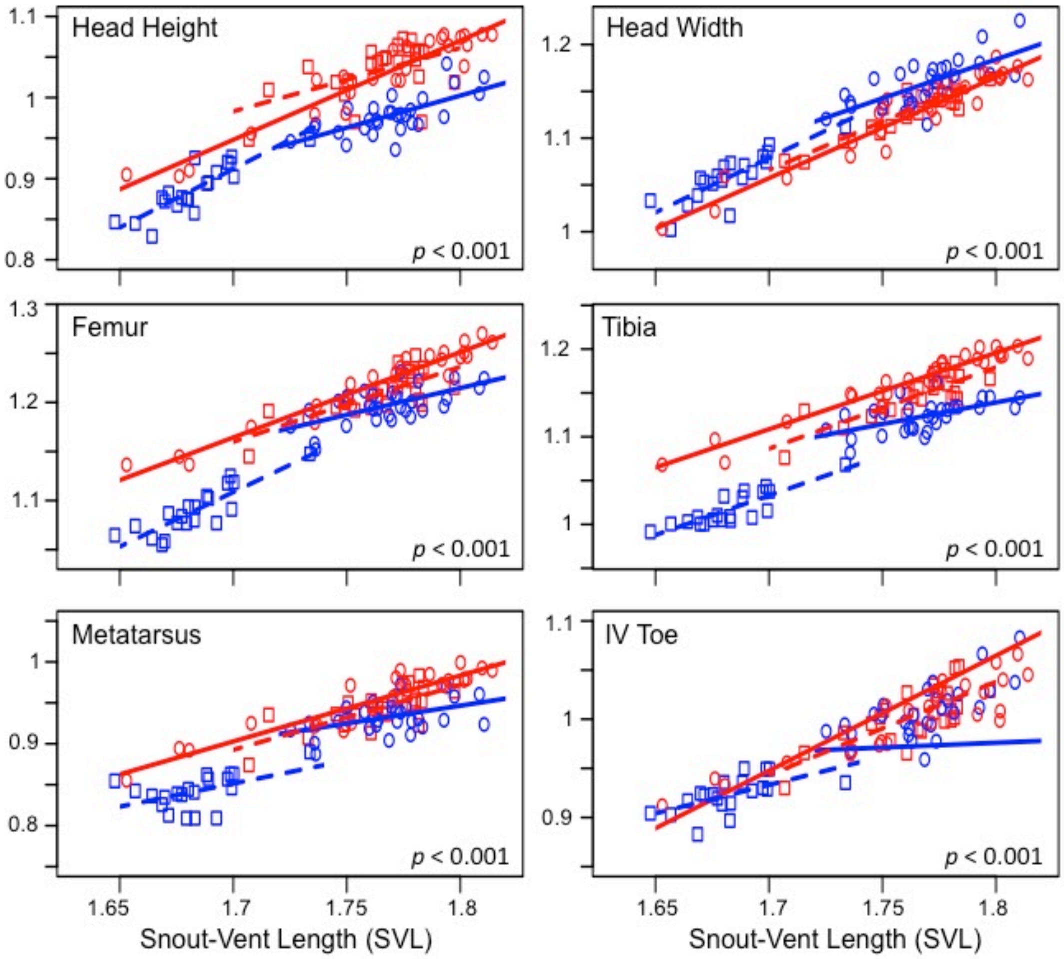

Figure 1_2. Morphological divergence between high (blue) and low elevation (red) *A. cybotes* lizards. Morphological variables are log-transformed. Squares denote lizards from the Sierra de Baoruco, and the regression line is given in dashed form. Circles denote lizards from the Cordillera Central and the regression line is solid. *p* values refer to test for differences in intercepts between high and low elevation populations. Details are given in Supp. Table 1_3.

understates the interactions occurring in nature. Because the niche is multidimensional, defined by the total of abiotic and biotic factors that determine where a species may persist (Hutchinson 1957; Holt 2009), there are potentially limitless dimensions that may be simultaneously influenced by behavioral shifts. For example, shifts in perch use also influence the prey organisms that lizards encounter, as well as their vulnerability to predators, to name only a few.

The interaction between behavioral drive and behavioral inertia may broaden our understanding of many evolutionary phenomena, such as niche conservatism. The tendency for ecological niches to be conserved over evolutionary time has been widely documented (Wiens et al. 2010), and presents an imminent threat to biodiversity as the climate continues to warm (Cahill et al. 2013; Quintero & Wiens 2013). By knowing which traits tend to be conserved, scientists are forecasting which behavioral changes, such as phenological shifts (Parmesan and Yohe 2003) or basking shifts (Kearney et al. 2009; Sinervo et al. 2010) are likely to occur. Appreciation for how behavioral shifts lead to interactions among traits can push this field forward by making it more predictive, as behavioral shifts to maintain homeostasis in the climatic niche are likely precipitating evolution along other trait dimensions. Thus, organisms' responses to climate change, and potentially even to other human-mediated selective pressures such as novel invasives, likely reflect a balance between behavioral drive and inertia. In short, the interaction between behavioral drive and behavioral inertia present an avenue for a more complete understanding of the complex role of behavior in evolution, and shed new insight into the evolutionary process.

Materials and Methods:

Study sites

We conducted our study at low and high elevation localities in each of the two main mountain chains of the Dominican Republic: the Cordillera Central in the east and the Sierra de Baoruco in the west (Supp. Fig. 1_1; Supp. Table 1_1). The occupation of highland environments occurred independently in the Cordillera Central and Sierra de Baoruco (Glor et al. 2003; Wollenberg et al. 2013). Study sites in the western mountains were located in Los Patos, Barahona Province (13 m.a.s.l.) and in Loma del Toro, Sierra de Baoruco National Park, Independencia Province (2258 m). Study sites in the east were located in the Francisco Alberto Caamaño Deñó National Park, Azua Province (43 m) and in Valle Nuevo National Park, La Vega Province (2450 m). Habitats near sea level are comprised of broadleaf hardwood tree species. In contrast, high elevation environments (> 2000 m.a.s.l.) consist of monodominant pine forests, as freezing temperatures preclude the expansion of broadleaf vegetation (Martin et al. 2011). Each site contained a mixture of trees and rocks.

Body Temperature And Habitat Use Measurements

We measured body temperature, T_b, from adult male lizards during continuous sampling periods (0600 – 1900) for six consecutive days at each locality, for a total of 312 sampling hours across all localities. We measured T_b in the Cordillera Central (Caamaño and Valle Nuevo) from June 3 – 8, 2012 and in the Sierra de Baoruco (Los Patos and Loma de Toro) from June 11 – 16, 2012. Following established methods (Hertz 1992; Muñoz et al. 2014), two researchers walked slowly through each habitat and captured adult lizards using a noose tied to the end of a pan fish pole. Males were considerably more conspicuous than females and juveniles, particularly in high elevation localities, where there were fewer animals overall. Thus we restricted our study to adult male lizards. Males were identified by the presence of an enlarged throat fan (dewlap) and enlarged post-anal scales (Schwartz 1989). For each lizard caught T_b was measured to the nearest 0.1°C using a thermocouple (Type T, Copper-Constantan) inserted approximately 1 cm into the cloaca and connected to a temperature logger (HH603A, Omega). In addition to T_b, we also recorded time of capture, perch substrate type and perch height (to the nearest 1 cm) for each lizard captured. Perch height was taken as the distance from the ground to the point where the lizards' snout was first observed. All lizards were marked semi-permanently using non-toxic ink to ensure that individuals were sampled only once.

Given that we were interested in understanding how vegetation and rocks differ in thermal quality and functional demands on lizards, we combined observations for lizards using all types of vegetation – branches, twigs, trunks, logs, and leaves – into a single 'vegetation' perch category. We used G-tests to determine whether perch choice differed among populations. Further, we also removed observations for lizards found on other substrates such as concrete walls and on the ground, which in total only accounted for 10.5% of total observations.

Thermal environment measurements

To collect information on the operative temperature (T_e) we deployed thermal sensors at each of our localities. By distributing models throughout a habitat, operative temperatures describe the temperatures non-thermoregulating lizards would attain (Bakken 1992). Briefly, following Bakken and Angilletta (2014), we built 44 electroformed copper models in the shape of an adult male *A. cybotes*, which we then painted to match the reflectance of live lizards. We embedded an iButton temperature sensor (DS1921G, Maxim) into each of the models, allowing for continuous temperature measurement. Further details on model design and calibration are given in Muñoz et al. (2014).

To ensure comparability of T_e and T_b, we deployed the copper models on the same days we sampled T_b from live lizards. At each site we placed models in roughly equal numbers along habitat transects. To build transects, we selected a starting point within each habitat and, using a random number table, determined the number of paces between model perches. We also used a random number table to select the height and orientation on trees at which we placed models using a random number table, such that numbers corresponded to perch heights varying from 0 – 150 cm in 15 cm increments and to orientations varying from 0° – 360° in 30° increments. We chose 150 cm as the maximum perch height for model placement because previous research has found that the vast majority of individuals of this species perch below this height (Fobes et al. 1992; Huyghe et al. 2007; Wollenberg et al. 2013). We switched model perches halfway through each sampling period to different perches to maximize our sampling of the thermal variation in each habitat. Models recorded T_e every ten minutes from 0600 to 1900 for six continuous days at each locality. On average we recorded T_e during 1,810 lizard model hours for each locality.

Analysis of thermal habitat and thermoregulatory behavior

We calculated the extent of behavioral thermoregulation, E, following Hertz et al. (1993) as follows:

$$E = 1 - \frac{\overline{d_b}}{\overline{d_e}}$$

where d_b and d_e refer to the mean deviation of T_b and T_e from the set-point range, respectively. The set-point range, or preferred temperature (T_p) range, refer to the central 50% of temperatures measured in a laboratory gradient (see below). Values of E approaching one signify less similarity between d_e and d_b and, correspondingly, denote greater thermoregulatory efficiency.

Physiological Measurements

We measured the preferred body temperature (T_p) of wild-caught, adult male lizards from all localities using laboratory thermal gradients. T_p refers to the mean temperature of a lizard when placed in a thermal gradient and allowed to choose where to sit; particularly in diurnal lizards such as anoles, T_p exhibits a strong positive correlation with the optimal temperature for performance (Huey et al. 2012). The experimental chamber consisted of a pine box (0.5” thickness) with eight identical lanes 91 cm long, 15 cm wide, and 14 cm tall which was covered by chicken wire. To create a temperature gradient, we laid a bed of sand and gravel over reptile heat cables (150 watts, Zoo Med). The gradient chambers were kept in an air-conditioned room, thus creating a stable range of operative temperatures ranging from 18°C – 40°C, which we confirmed before each experiment. To measure lizard temperature during the preferred temperature experiments, we inserted an Omega temperature probe (Type T, Copper-Constantan, 36 gauge) approximately 1 cm into the cloaca of each lizard and secured it to the base of the tail using a small piece of medical tape. The temperature probe was connected to a digital temperature logger (HH147U, Omega) that recorded temperature every 10 minutes during the experiment. We let lizards acclimate to the experimental chamber for 30 minutes prior the beginning of the experiment. Temperature loggers recorded T_b every 10 minutes during each 4-hour experimental period. We estimated T_p as the mean of the central 50% of T_b measurements (following Huey 1982; Hertz et al. 1993) measured during the experiment. We compared T_p among populations using ANOVA.

We measured heat tolerance, CT_{max}, in adult male lizards from each of the populations sampled. CT_{max} refers to the temperature at which a lizard fails to right itself when flipped onto its back (Spellerberg 1972). The methods for measurement of CT_{max} is given in Muñoz et al. (2014). Briefly, we placed an Omega temperature probe (Type T, 36 gauge) approximately 1 cm into the cloaca of an adult lizard as described above. Each lizard was placed into a perforated Tupperware container, where it was allowed to reach ambient temperature. The experimental chamber consisted of 100 W light bulb suspended approximately 30 cm above an insulated box. The Tupperware was placed in the insulated container and temperature was raised at a rate of ~1°C/min until the panting threshold was reached. This threshold denotes the point at which lizards begin to cool via evaporative cooling by opening their mouths (Hertz et al. 1979). At this point, we flipped lizards onto their backs and encouraged them to flip over by gently stimulating the base of its tail and thighs using a pair of blunt tweezers. When lizards flipped within 15 seconds, we raised the temperature 0.5°C more and repeated the procedure, until a temperature was reached at which lizards failed to right themselves, which we considered CT_{max}.

Morphological Analysis

We restricted our morphological analyses to traits known to be of ecological importance for adaptation to a rock-dwelling lifestyle in lizards. Multiple studies have demonstrated that rock dwelling lizards tend to exhibit flatter, wider heads (e.g., Revell et al. 2007; Goodman et al. 2008). Thus, we predicted that lizards at high elevation should have flatter, wider heads than at low elevation.

We collected adult male lizards from each of the four sampling localities. Body length was measured as the distance from the tip of the snout to the cloaca; head height was measured as the distance from under the base of the jaw (angular) to the top of the head (parietal); head width was measured as the distance between the quadratojugal processes of the jugal. Hindlimb dimensions included femur length, tibia length, metatarsal IV length, and hindtoe IV length.

SVL and head height were measured with calipers (Mitutoyo) to the nearest 0.01 mm and lamella number was counted using digital images (1200dpi) generated using a flatbed scanner. All other traits were measured using digital images generated from a portable X-ray machine (Kodex) using the Object-J plugin in Image-J ver. 1.47 (Abramoff et al. 2004). All values were measured twice and the average was used for subsequent analyses. A single investigator (M.M.M.) performed all measurements. We performed statistical analyses on log-transformed morphological variables. We tested for significant morphological differences between high and low elevation lizards for each individual trait using analysis of covariance (ANCOVA) with elevation as fixed effects and body size (SVL) as a covariate.

Common Garden Experiment

In July 2012 we collected gravid females from the high- and low-elevation western populations and kept them in enclosures with one adult male from the same population. Each enclosure consisted of a 12”x12”x12” polyester/nylon mesh cage (BioQuip Products) with a felt cage carpet, 2 bamboo dowels for perching, and a small pot with moist soil for laying eggs. Cages were misted twice daily and illuminated with full spectrum lighting. Lizards were fed 0.5” vitamin-dusted crickets 3 times per week.

Cages were checked 3-4 times per week for eggs. Only two eggs were laid by females from the high elevation population (Loma de Toro) under our laboratory conditions and no changes in humidity, temperature, or light cycle that we tried could induce the females to become gravid again, despite several months of effort. Thus, we chose to excise eggs from females from this population. In June 2013 we collected heavily gravid females (determined through visual inspection and ventral palpation), which we decapitated using large surgical shears immediately removed the shelled eggs from the oviduct.

When we found eggs were found in a cage or extracted them from a dam, we immediately transferred them to cell culture dishes containing moistened vermiculite. We found that eggs from the high elevation population rapidly desiccated when kept in a standard incubator, even when frequently misted, and so we incubated all eggs at room temperature (23.5°C). Incubation dishes were misted to keep vermiculite moist as needed, and were checked daily for hatchlings. Hatchlings were immediately transferred to individual Critter Keeper cage with a mesh lid that contained a felt substrate, wooden dowel, and a small plant. The environmental chambers were kept at 29.4°C, and followed a 12:10 light:dark cycle using full spectrum bulbs, and we misted cages twice daily. We fed juvenile lizards vitamin-dusted *Drosophila* twice weekly until they reached approximately 20 mm in body size (SVL), at which point they were switched to a diet of vitamin-dusted crickets (0.25" – 0.5"). Cages were misted twice daily. Lizards were reared to adulthood (8 – 12 months). As with adults, we compared each morphological among high and low elevation lizards using ANCOVA.

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Chapter 2

Behavioral drive, behavioral inertia, and the Levins-Lewontin Effect

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Abstract

How organisms interact with their environment is an important pacemaker for evolution. It has long been recognized that behavior can impel (*behavioral drive*) and impede (*behavioral inertia*) evolution. Here we explore the idea that behavioral inertia is accomplished through behavioral drive along different niche dimensions, a phenomenon we term the 'Levins-Lewontin Effect'. We provide an empirical example of the Levins-Lewontin Effect by showing that physiological stasis in montane populations of a tropical lizard necessitated a behavioral shift that impelled morphological divergence. We examine the Lewontin Effect in the context of niche conservatism (NC), which is an emerging principle in evolutionary biology, through a literature survey. We find a multidimensional approach to the study of niche evolution can elucidate connections and interactions among niche dimensions. Knowledge of the interplay between behavioral inertia and behavioral drive can better inform predictions for how organisms will respond to contemporary selective pressures.

The ‘Levins-Lewontin Effect’

The relationship between organisms and the environment has long been thought unidirectional: environments set demands that determine which individuals have greater fitness. In reality, however, organisms are not passive vessels at the whim and mercy of the environment. Rather, through behavior, organisms alter how they interact with the environment through shifts in resource utilization, activity patterns, and structural habitat use, among many others (Lewontin 1983; Levins and Lewontin 1985; Huey et al. 2003). As a result, behavior has the power to greatly influence the pace and magnitude of evolutionary divergence (West-Eberhard 1989; Price et al. 2003; Duckworth 2009).

There are two hypotheses for the role of behavior in the evolutionary process. The ‘behavioral drive’ hypothesis as proposed by Ernst Mayr (1959, 1963) posits that behavior impels evolutionary change. Mayr argued that, by allowing organisms to interact with the environment in a novel way, behavioral shifts expose them to new selective pressures, in turn accelerating evolution. The ‘behavioral inertia’ hypothesis, first articulated by Charles Bogert (1949), argues posits that some behaviors, such as thermoregulation, enable organisms to experience the same selective environment even as they colonize new environments or conditions change in their ancestral habitats. By buffering organisms from novel selective pressures, ‘inhibitory’ behaviors may preclude or reduce evolutionary change, a phenomenon also referred to as the ‘Bogert Effect’ (Huey et al. 2003).

It has long been recognized that these two phenomena, ‘behavioral drive’ and ‘behavioral inertia’, are not mutually exclusive. For example, behavior can shield organisms from selection on short time scales following a shift to a novel habitat, while also driving adaptation over longer time periods (West-Eberhard 2003; Duckworth 2009). Or, as Lapiedra et al. (2013) found, behavior shifts can promote the evolution of novel morphological structures, which are then subject to stabilizing selection, thus inhibiting the evolution of reversions.

However, a factor that has often been overlooked is that behavior can have multiple, possibly competing effects on evolution in different niche dimensions. In *The Dialectical Biologist*, Levins and Lewontin (1985) argued that organisms and their environments interact to shape the selective pressures that they experience. The same behavior can exert different selective pressures on the same traits, leading to tradeoffs in selective pressures among different niche dimensions. For example, Lewontin (1983) points out that habitat selection is a key aspect of a lizard's dietary niche, but habit selection is also equally linked to its climatic niche. Thus, shifts in habitat selection that shield organisms from selection in one niche aspect may necessarily expose them to selection on another. Their insight is that, through behavior, organisms define their environments and these interactions influence whether and how different traits evolve.

In light of these ideas, Huey et al. (2003) proposed that this interaction among niche dimensions was intrinsically linked to behavioral drive and behavioral inertia. Specifically, they proposed that behavioral shifts that achieve homeostasis in one niche dimension inevitably occurs through non-homeostasis along another. Though it is nearly three decades old, the idea that behavioral inertia necessarily precipitates behavioral drive has received almost no attention, though it has wide implications for evolutionary biology. In the subsequent section, we give an empirical demonstration of the Lewontin Effect from our own work on the tropical lizard, *Anolis cybotes*. We then examine the

Lewontin Effect in the context of niche conservatism, and use a literature search to highlight promising avenues for future research.

An empirical demonstration of the Levins-Lewontin Effect

The Caribbean lizard *Anolis cybotes* is found nearly island-wide on Hispaniola. Though it is a tropical island, Hispaniola harbors extensive highland habitat (*sensu* Wollenberg et al. 2013), leading to greater topographic and environmental heterogeneity than elsewhere in the Caribbean. Consequently, *A. cybotes* is found in habitats as divergent as xeric lowland scrub and montane pine forest, and environments that differ in mean annual temperature by as much as 15°C.

Ectotherms such as lizards can adjust to different thermal habitat either through behavioral shifts that shield them from thermal variation across habitats, or through physiological adaptation to different environmental temperatures. These two possibilities lead to two mutually exclusive hypotheses for physiological evolution in *A. cybotes* – if the former is true, we expect high elevation populations to exhibit similar preferred body temperatures as their low elevation counterparts (i.e., the 'Bogert Effect'). If the latter is true, however, then high elevation lizards should have lower preferred body temperatures than lizards near sea level.

We found strong evidence for the Bogert Effect – despite marked differences in environmental temperatures (T_e), mean body temperatures were nearly identical among populations separated by almost 2,400 meters (Fig. 1A). Correspondingly, we found that

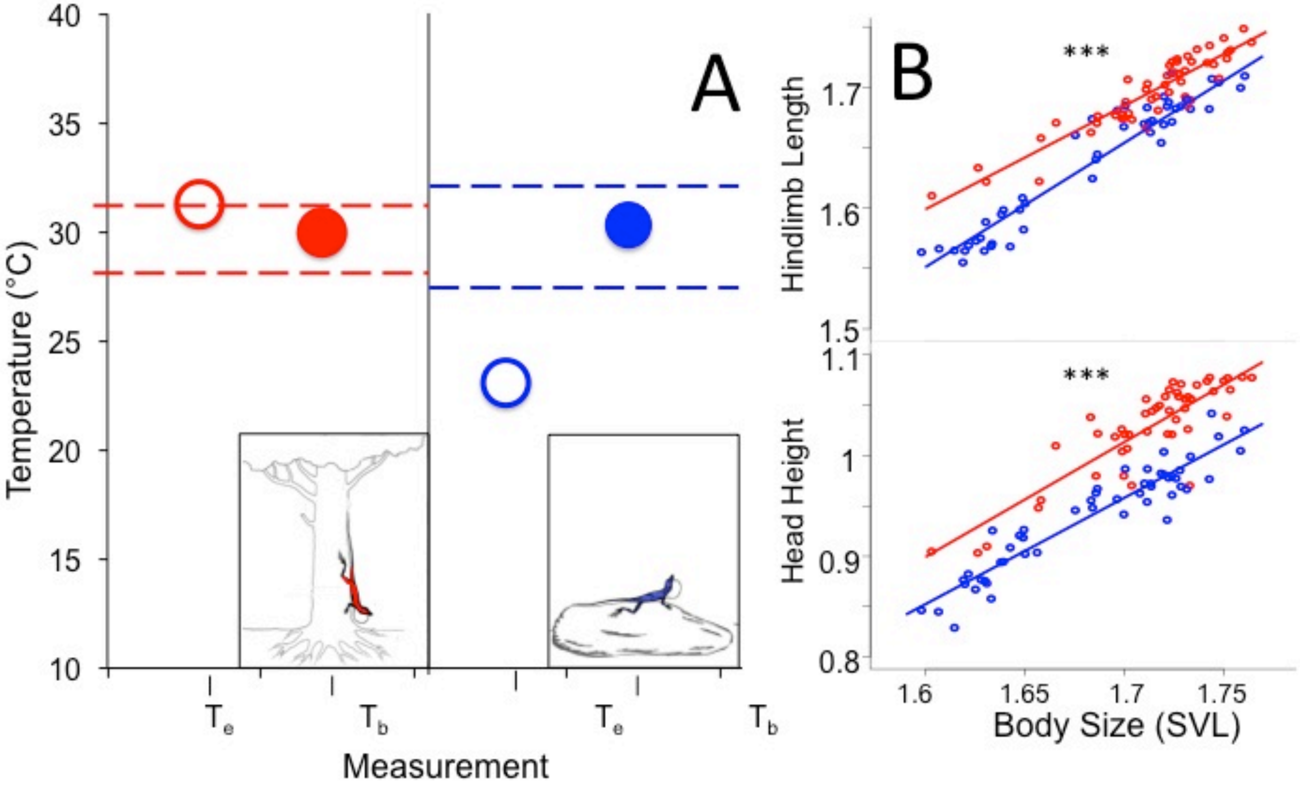

Figure 2_1. (A) The mean environmental temperatures (operative temperature; T_e) for high (2385 m – blue) and low (50 m – red) elevation localities are given in open circles, whereas mean lizard body temperature (T_b) is given in closed circles. The dashed lines denote the preferred temperature range (T_{set}). The inset depicts the typical perching behavior of *A. cybotes* at different elevations. (B) Morphological differentiation between high and low elevation populations of *A. cybotes*. Body size (snout-vent length; SVL) is plotted

Figure 2_1 continued: against hindlimb length (top panel) and head height (bottom panel). All morphological variables are log transformed. In both cases, high and low elevation populations have significantly different intercepts, but not slopes. For both (A) and (B) results from two transects are combined to show the full high versus low comparison. Results from transects were identical.

the preferred body temperature, which correlates with the optimal temperature for performance, was inert among populations. Thus, behavioral thermoregulation is strongly associated with physiological stasis, suggesting that the Bogert Effect is occurring in *A. cybotes*.

We found that thermoregulation is achieved through a behavioral shift in substrate use. Lizards at low elevation were nearly always perching on shady arboreal vegetation whereas high elevation lizards were consistently found basking on open rocky substrates (Fig. 2_1A inset). Trees were nearly always too cold for lizards at high elevation, whereas rocks were lethally hot throughout most of the day at low elevation – thus, shifts in substrate use confer a distinct thermoregulatory advantage.

In the case of *A.* cybotes, behavioral inertia is achieved through shifts in resource use. In this example, a shift in substrate at high elevation used to remain in the same climatic niche involved a shift in the habitat niche. If the Levins-Lewontin Effect is occurring, the shift to rocks precluding physiological evolution should impel evolution along a different dimension. Consistently, we found that the use of rocks and boulders promoted the evolution of flatter heads and shorter hindlimbs in high elevation lizards (Fig. 2_1B). Thus, behavioral inertia leads to physiological stasis and induces behavioral drive through morphological divergence. As Levins and Lewontin (1985) and Huey et al. (2003) predicted, stasis along one niche dimension is purchased through divergence along another.

The Levins-Lewontin Effect as a lens for niche conservatism

The idea that stasis in one niche dimension can only occur through divergence along another has potentially broad implications for a variety of evolutionary phenomena, such as niche conservatism. Niche conservatism (NC) refers to the tendency for ecological niches to be conserved over evolutionary time (Peterson et al. 1999). Although this concept dates back at least three decades, it has recently exploded as a line of research, particularly since the publication of an influential review by Wiens and Graham (2005; Fig. 2_2). NC is a useful heuristic for understanding many phenomena in evolutionary biology, including speciation, community assembly, and diversity and species richness gradients (Wiens and Graham 2005; Wiens et al. 2010).

The generality of this concept, however, currently remains uncertain, with some arguing that divergence among populations typically occurs in the absence of ecological differentiation (Peterson et al. 1999), and other contending that niche shifts are critical for diversification (e.g., Losos et al 2003; Graham et al. 2004). Some have rightly argued that NC represents a continuum, and that niche divergence (ND) as well as niche stasis both frequently occur in nature (Wiens et al. 2010; Peterson 2011).

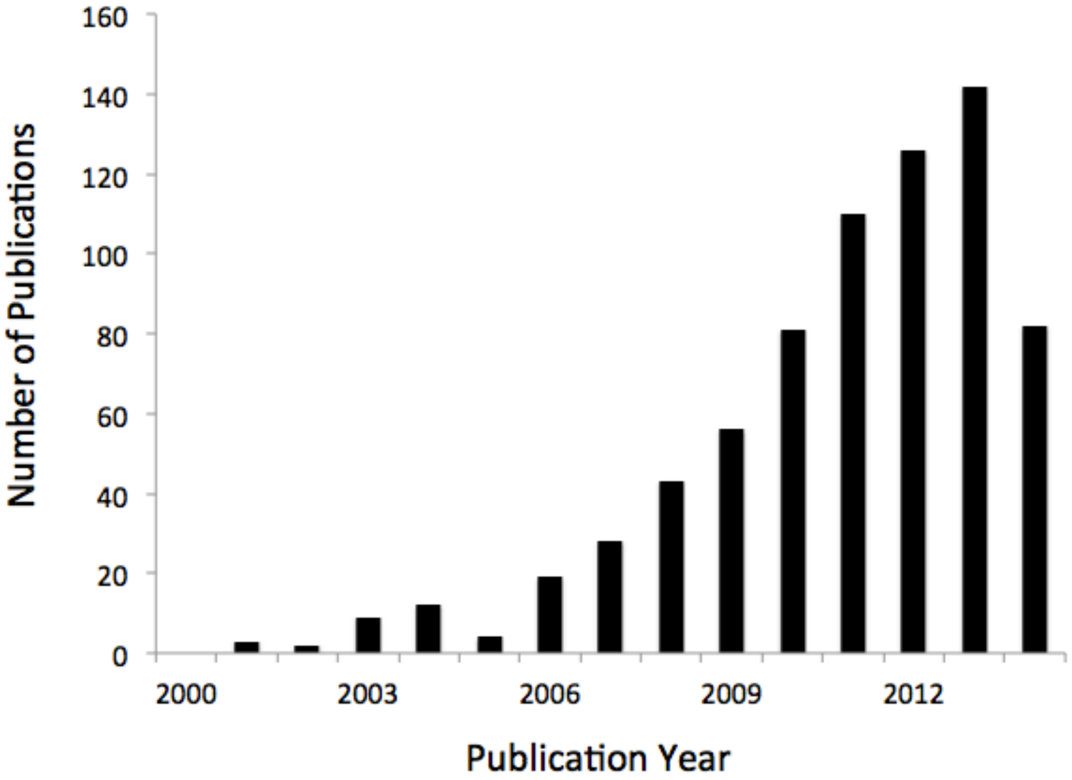

Figure 2_2. Histogram showing the number of publications considering NC between 2000 and the present. The literature search was performed using the term “niche conservatism” on the ISI Web of Knowledge on August 13, 2014 and yielded 717 results since 2000.

In light of the Levins-Lewontin Effect, we argue that a comparatively unexplored factor influencing support for NC is that niches are likely simultaneously static and labile along different dimensions. That is, instances of NC in one niche dimension are likely matched with ND along another, and vice versa. We argue that this diversity of pattern does not simply reflect a diversity of process – rather, different aspects of an organism’s ecology are linked through shared resource use, leading to NC and ND concomitantly. We use a literature search to explore this idea.

Our goal was to assess the number of niche axes and study outcome (evidence for niche conservatism, niche divergence, or both) in studies examining NC. Certainly the evidence for NC has been critically assessed before. In a literature survey assessing empirical support for phylogenetic signal (λ), which measures the tendency for traits to phylogenetically correlated among taxa, Freckleton et al. (2002) found that the phylogenetic dependence of trait evolution was strongly supported. Subsequent reviews by Losos (2008) and Pearman et al. (2008) found that support for NC is equivocal, whereas Peterson (2011) found that NC is a common phenomenon, particularly at shallow phylogenetic scales. To our knowledge, however, ours is the first review to explicitly consider how the number and types of niche dimensions examined affect the support for NC.

Although organisms interact with their environments in potentially limitless ways (Hutchinson 1957; Holt 2009), most of the variation in a species' niche can be summarized as distributions in resource utilization along four principal axes: climate, diet, habitat, and time (modified from Pianka 1969; Schoener 1974, 1989). The climatic niche encompasses the climatic envelopes that determine where species may persist. Dietary utilizations refer to the number, size, and types of different prey items. Habitat use refers to the portion of the structural habitat the organism utilizes. Finally, temporal utilizations refer to organisms' daily or seasonal activity patterns.

To assess the number and types of niche dimensions in NC studies we examined empirical studies on the topic published since the 2005 review by Wiens and Graham to the present day. We searched all papers published from 2005 onwards using the ISI Web of Science database and the search terms ‘niche conservatism’. Of the 717 results, we examined in detail those that were empirical works or meta-analyses of empirical works examining niche evolution. Specifically, we considered empirical studies in which the authors explicitly quantified and compared niche aspects within or among species. We did not consider studies examining NC in the context of phenomena unrelated to the process of niche evolution, such as phylogenetic community assembly (e.g., Cavender-Bares et al. 2006; Swenson et al. 2007) or the heritability of geographic range size (e.g., Mouillot & Gaston 2007). We further expunged studies that utilize organismal traits as proxies for niche characteristics because such traits may reflect adaptation to various niche axes, or to none at all. Following Schoener (1989), we scored the number and types (climate, diet, structural habitat, and time) of niche axes examined for all of the remaining studies. Rather than offer interpretation, especially since studies vary greatly in the null tests that they utilize (Warren et al. 2008), which complicates comparing them, we noted whether the authors found that their results were consistent with niche conservatism, niche divergence, or both.

The first observation from our survey is that most studies most studies (87%) examine only a single niche dimension and 92% of those focus on climatic niche evolution (Table 2_1). Among unidimensional studies the support for NC was mixed, with more 55% finding support for niche retention and 26% finding support for niche divergence. A smaller fraction of these studies (19%) found support for both divergence and conservatism. These studies were typically conducted at larger phylogenetic or geographic scales, or both (Supp. Table 2_1), allowing for a mixture of patterns across different lineages or geographic regions, a tendency that has been noted elsewhere (Peterson 2011). Another possibility is that different aspects of each niche dimension exhibit different patterns and that our breakdown of niche axes are overly simplistic. For example, precipitation and temperature, though aspects of the climatic niche, may

Table 2_1. Number and types of axes (climate, diet, habitat, time, and combinations among factors) examined in NC studies since 2005. Niche dimensions are based on Schoener (1989) and described in the text.

Single Axis					
Climate	Diet	Habitat	Time		
72	0	6	0		
Outcome					
Conservatism	Divergence	Both			
43	20	15			
Multiple Axes					
Climate, Diet	Climate, Habitat	Climate, Time	Climate, Diet, Habitat	Climate, Time	Habitat, Time
1	4	3	2	1	1
Outcome					
Conservatism	Divergence	Both			
1	3	8			

influence different patterns of niche evolution. Nonetheless, the majority of unidimensional studies provide only a single outcome for niche evolution. Most undimensional studies find evidence for NC, but because divergence is only examined along a single axis, it is impossible to tell whether such conservatism occurs through divergence along another axis.

A much smaller fraction (13%) of studies considered niche evolution along at least two dimensions. Of the studies that examined more than one niche dimension, eight examined niche evolution along two axes, and three examined evolution along three axes (Table 2_1). Most of the multidimensional studies (67%) found evidence for both niche divergence and niche conservatism, although a handful of studies found that their results were consistent with only divergence or stasis. Thus, though the trend is not universal, most multidimensional studies that find stasis in one dimension also find evidence for divergence along another.

Although our findings are not confirmatory, they are largely consistent with the Levins-Lewontin Effect. A deeper examination of the multidimensional studies reveals the potential for the Levins-Lewontin Effect to be influencing patterns of niche conservatism and divergence. For example, in a study of flowering time in native and introduced populations of various plant species, Hulme (2011) found that plants were tracking their climatic niche through predictable phenological shifts. Other studies also reveal this link between flowering phenology and climatic niche conservatism (Alexander 2013; Amano et al. 2014). Stasis in the climatic niche achieved through shifts in phenology can lead to novel ecological interactions through the presence of novel pollinators, predators, and parasites, for example, and thus precipitate evolutionary change. In order to determine whether a tradeoff exists along niche dimensions, however, further work is necessary to establish the behavioral connection between shifts in resource use and niche evolution.

Our perspective highlights implications for the understanding of niche evolution in the context of contemporary selective pressures, such as climate warming, habitat modification, and novel invasives. Understanding which aspects of niches tend to exhibit conservatism can inform predictions for evolutionary divergence in other traits. Above we discussed how the Levins-Lewontin Effect can lead to evolutionary divergence in response to climatic niche conservatism, but the same effect likely holds for other types of niche conservatism. When behavioral shifts accommodate ancestral niche conditions, selection may promote divergence in other niche axes through novel ecological interactions or the availability of new resources (Schoener 2011). Deeper understanding of the behavioral link between resource use and niche evolution can lead to more predictive studies of contemporary evolution.

Concluding Remarks

Behavioral shifts can simultaneously impede and impel evolution – in fact, it is likely that in many cases behavioral inertia requires behavioral drive. In the case of *Anolis cybotes* the Levins-Lewontin Effect occurs through behavioral inertia precluding physiological evolution, whilst concurrently driving morphological evolution. The behavioral link between resource use distributions informs the process of niche evolution: shifts in resource use that reduce selection along one dimension shift the selective regime on traits relevant to a different dimension. In short, a multidimensional perspective on niche evolution would elucidate the interplay between ecological dimensions and their effect on evolution; these perspectives could lead to clearer predictions for how organisms may respond to pernicious contemporary selective pressures.

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Chapter 3

Evolutionary stasis and lability in thermal physiology in a group of tropical lizards.

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Abstract

Understanding how quickly physiological traits evolve is a topic of great interest, particularly in the context of how organisms can adapt in response to climate warming. Adjustment to novel thermal habitats may occur either through behavioral adjustments, physiological adaptation or both. Here, we test whether rates of evolution differ among physiological traits in the cybotoids, a clade of tropical *Anolis* lizards distributed in markedly different thermal environments on the Caribbean island of Hispaniola. We find that cold tolerance evolves considerably faster than heat tolerance, a difference that results because behavioral thermoregulation more effectively shields these organisms from selection on upper than lower temperature tolerances. Specifically, because lizards in very different environments behaviorally thermoregulate during the day to similar body temperatures, divergent selection on body temperature and heat tolerance is precluded, whereas night-time temperatures can only be partially buffered by behavior, thereby exposing organisms to selection on cold tolerance. We discuss how exposure to selection on physiology influences divergence among tropical organisms and its implications for adaptive evolutionary response to climate warming.

Introduction

Rising temperatures present unique challenges for tropical ectotherms, which already generally function near their upper thermal limits: even small temperature increases can have disproportionately large negative consequences for these organisms (Deutsch et al. 2008; Huey et al. 2009). Studies assessing tropical ectotherms' vulnerability to climate warming have traditionally focused on predicting where warming will have the most pronounced effects on organismal fitness by correlating physiological traits with environmental data and using these relationships to infer where range shifts and local extinctions will occur (e.g., Sinervo et al. 2010; Chen et al. 2011). However, the evolutionary potential of populations to respond to novel selective pressures imposed by changing climate is an equally important and comparatively unexplored aspect of response to climate warming (Williams et al. 2008).

Behavior and physiology can interact to determine how organisms mitigate their thermal environments. Organisms that thermoregulate limit exposure to suboptimal temperatures – a phenomenon commonly referred to as the 'Bogert effect' (Bogert 1949; Stevenson 1985; Huey et al. 2003). Physiological traits that behavioral thermoregulation can shield from selection should exhibit less evolution than traits that cannot be so easily buffered and thus are exposed to stronger selection. As a given environment is more thermally complex in the day than at night (Sarmiento 1986; Ghalambor et al. 2006), thermoregulation should be more effective at shielding organisms from selection on upper than lower physiological limits and, consequently, tolerance to cold should evolve faster than optimal performance temperature and tolerance to heat.

In this study we compare rates of physiological evolution in the cybotoids, a tropical clade of *Anolis* lizards from the Caribbean island of Hispaniola whose members differ extensively in thermal habitat (Schwartz 1989; Glor et al. 2003). Previous work by Hertz and Huey (1981) found similar body temperatures and heat tolerance among three species of the cybotoids and provided ecological data suggesting they are good thermoregulators. Our study expands on this work in terms of populations, species, and physiological traits examined. The cybotoid clade is unique among Caribbean anoles because its species are found from sea level to almost 3,000 meters (Henderson and Powell 2009), and the environments they experience pose different selective pressures and provide the opportunity for behavioural and physiological adaptation 70 to different thermal extremes.

We first present a comparative analysis of three ecologically important physiological traits – cold tolerance (CT_{mix}), body temperature (T_b), and heat tolerance (CT_{max}) – across thermal environments. We then compare rates of evolution among these traits using a likelihood-based approach and test whether behavioral thermoregulation can limit exposure to extreme temperatures using field estimates of basking site selection and night-time temperatures. Finally, we discuss the role of behavior in mediating physiological evolution in tropical ectotherms and how behavior influences adaptive evolutionary potential in physiological traits.

Materials and Methods

Study organisms and study sites

The cybotoid anoles are a clade of nine species from Hispaniola commonly found on trunks or near the ground (Schwartz 1989; Glor et al. 2003). Our sampling was conducted in June and July 2011 and focused on the seven cybotoids found in the Dominican Republic (Fig. 3_1). The other species are *A. breslini*, which is restricted to northwestern Haiti and is ecologically quite similar to *A. whitemani* (Williams 1963), and *A. haetianus*, which is found only in Western Haiti and likely represents a morphological variant of the widespread *A. cybotes* (Glor et al. 2003). Where possible, we sampled

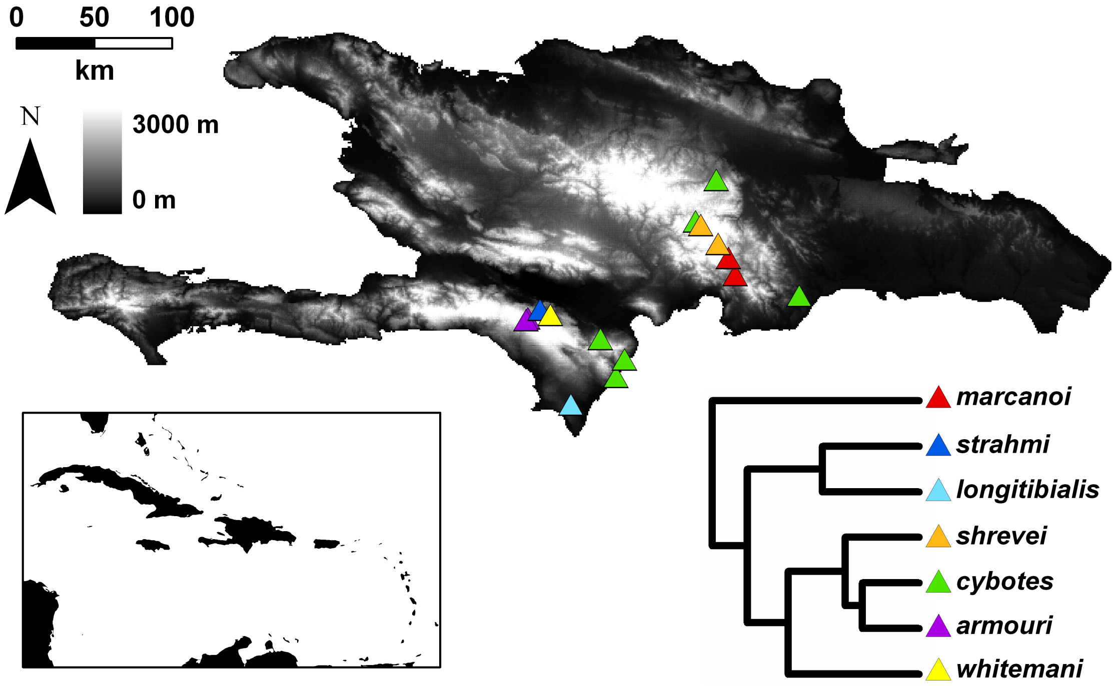

Figure 3_1. Map showing altitudinal variation and the localities for each cybotoid population sampled in this study. The map inset shows the location of Hispaniola with respect to the other islands in the Caribbean basin. Greyscale indicates elevation and range from dark (low) to light (high) elevation. Species are denoted in different colors and shapes in the phylogeny generated based on Mahler et al. (2010).

several populations that, together, encompassed most of that species' altitudinal range (Fig. 3_1; Table 3_1). *Anolis cybotes* is found nearly island-wide; we sampled it at three elevations in each of the two principal mountain chains, the Sierra de Baoruco (SB) and Cordillera Central (CC). In the SB we sampled the mid-elevation species, *A. strahmi* and *A. whitemani*, and the high elevation species, *A. armouri*. In the CC, we sampled the mid-elevation species *A. marcanoi*, and the high elevation species, *A. shrevei*.

We gathered climatic measurements for each locality by extracting all temperature variables (bio 1 – bio 11; Supp. Table 3_1) from environmental layers available in the WorldClim dataset (resolved to approximately 1km^2; Hijmans et al. 2005). These variables summarize seasonal and annual temperature trends. To account for collinearity among thermal variables, we reduced data dimensionality using a principal components (PC) analysis on the correlation matrix.

Measurement of physiological performance indices

We measured the critical thermal minimum (CT_{min}) and maximum (CT_{max}), which refer to the low and high temperatures at which an organism loses locomotor function; these are widely used for measuring the tolerance limits of performance in ectotherms (Spellerberg 1972; Lutterschmidt and Hutchison 1997). We measured tolerance range, which refers to the range at which organisms can move (Huey and Stevenson 1979), as the difference between CT_{min} and CT_{max}. We also measured mean field body temperature (T_b).

Table 3_1. Locality name, species sampled, and altitude (m) are given. Mean CT_{min}, T_b, and CT_{max} are given. Units for physiological metrics are °C ± 1 s.e.m. and sample size is given in parentheses. CC: Cordillera Central; SB: Sierra de Baoruco.

Species	Locality	Alt (m)	CT_{min}	T_b	CT_{max}
Wild-measured					
A. cybotes (SB)	Los Patos	45	11.3 ± 0.4 (16)	30.1 ± 0.3 (45)	39.5 ± 0.1 (20)
*A. cybotes (*CC)	San Cristóbal	56	11.4 ± 0.2 (16)	29.4 ± 0.5 (53)	39.2 ± 0.2 (16)
A. longitibialis	Jaragua	105	12.8 ± 0.2 (18)	28.9 ± 0.2 (101)	38.5 ± 0.3 (18)
A. whitemani	Puerto Escondido	411	12.2 ± 0.4 (15)	27.9 ± 1.1 (17)	38.8 ± 0.3 (15)
A. strahmi	Camino Aguacate	454	11.3 ± 0.5 (6)	26.3 ± 0.7 (7)	39.2 ± 0.2 (6)
A. marcanoi	Ocoa	458	12.6 ± 0.3 (9)	–	38.2 ± 0.3 (9)
A. cybotes (CC)	Jarabacoa	690	10.7 ± 0.2 (18)	29.0 ± 0.6 (39)	40.3 ± 0.2 (18)
A. cybotes (SB)	Guayuyal	727	10.8 ± 0.4 (15)	26.6 ± 0.4 (53)	38.7 ± 0.2 (15)
A. marcanoi	La Horma	879	11.3 ± 0.1 (16)	29.1 ± 0.6 (48)	38.7 ± 0.2 (16)
A. cybotes (CC)	Constanza	1390	10.0 ± 0.5 (11)	29.2 ± 0.5 (10)	39.5 ± 0.6 (11)
A. cybotes (SB)	La Hoz	1395	8.7 ± 0.4 (9)	28.6 ± 0.9 (11)	38.9 ± 0.2 (9)
A. shrevei	Valle Nuevo – low	1950	9.6 ± 0.6 (9)	28.0 ± 1.0 (10)	39.9 ± 0.3 (9)
A. armouri	Loma de Toro – low	2020	8.2 ± 0.6 (9)	–	39.3 ± 0.3 (9)
A. armouri	Loma de Toro – high	2318	7.2 ± 0.4 (12)	25.9 ± 0.7 (21)	–
A. shrevei	Valle Nuevo – high	2450	6.2 ± 0.3 (11)	27.4 ± 1.0 (20)	40.4 ± 0.3 (11)
Acclimated					
A. cybotes	Los Patos	45	10.6 ± 0.3 (19)	–	–
A. armouri	Loma de Toro – high	2318	7.2 ± 0.3 (18)	–	–

We measured field body temperatures (T_b) during one continuous 13-hour period (0600 – 1900 hr) at 13 localities from 20 June to 31 July 2011. Due to logistical constraints, one locality (*A. shrevei* – 1950 m) was sampled from 0600 - 13:45, but results for that population are consistent with those from other populations (Table 3_1). Following established methods (Huey 1974; Hertz 1992), we conducted random sweeps of the habitat and captured adult male lizards with a noose. For every lizard, we recorded core temperature (T_b) to the nearest 0.1ºC using a thermocouple (Type T, Copper-Constantan) inserted ~1cm into the cloaca and connected to a temperature logger (HH603A, Omega) and measured each lizard only once. T_b generally correlates closely with the optimal performance temperature (T_{opt}, or the temperature at which organisms maximally perform a function such as running) in many diurnal lizards, including tropical anoles (Huey et al. 2012).

For each lizard captured, we recorded the time, weather conditions (sunny, mixed, or overcast skies), and "basking status" (basking in the full or filtered sun, perching in the shade under sunny or mixed skies, or in the shade under overcast skies) following Hertz (1992). For a statistical analysis of basking site choice, we removed observations for lizards captured in overcast conditions, as lizards did not have the opportunity to choose between perching in the sun or in the shade. We tested for weather-dependent basking choice using a logistic regression model such that individuals' basking behavior (perching in shade = 0, perching in sun = 1) was evaluated as a function of elevation, weather (sunny or mixed skies), and an elevation × weather interaction. An effect of elevation alone would indicate that certain basking behaviors are more likely to occur at different elevations, whereas a significant interaction would indicate that active lizards differ in how they exploit weather conditions at different elevations.

We measured CT_{min} and CT_{max} in adult males from each of the populations sampled. CT_{min} and CT_{max} are estimated as the temperature at which a lizard fails to right itself when flipped onto its back (Spellerberg 1972; Lutterschmidt and Hutchison 1997). After capture, we gave animals a 24-hour rest period in a large, insulated cooler (Coleman) in which temperature was maintained near 23°C at all sites. To measure core temperature during the tolerance experiments, an Omega temperature probe (Type T, 36 gauge) was placed approximately 1 cm into the cloaca of an adult lizard and secured to the base of the tail using a small piece of surgical tape, ensuring that tail movement was uncompromised. The temperature probe was connected to a digital temperature logger (HH147U, Omega). The lizard was placed into a perforated plastic container where it could move freely. After the lizard attained ambient temperature, the container was moved to an insulated icebox coated with a layer of crushed ice. Because the rate of temperature change during a tolerance experiment can alter an organism's performance (Terblanche et al. 2007), we endeavored to reduce body temperature at a constant rate of 1°C/min for all lizards. To conduct the experiment we reduced body temperature to 14°C, at which point we flipped the lizard onto its back using a pair of blunt tweezers and stimulated it to flip itself back over by gently probing the base of its tail and pressing its thighs. If the lizard flipped over after 15 seconds, then we lowered core temperature 0.5°C and tried again, repeating this procedure until a temperature was reached at which the lizard failed to right itself in the allotted time. CT_{min} was recorded as the temperature at which the righting response was lost.

Animals were given 24 hours in the insulated cooler to rest before CT_{max} trials.

The method for estimating CT_{max} was similar to that of CT_{min} except that a 100 W light bulb was suspended approximately 30 cm above the insulated container. We placed lizards in a breathable Tupperware container and increased their core temperature at a rate of ~1°C/min by exposing them to the heat source. We began flipping lizards when they began to cool through panting (i.e., the 'panting threshold'; Hertz et al. 1979) following the procedure described above, and recorded the temperature at which the right response was lost as CT_{max}.

Estimation of CT_{min} and CT_{max} is potentially confounded by the rate of temperature change, body size, and starting conditions (Gaston and Spicer 1998; Terblanche et al. 2007; Chown et al. 2009). We performed linear regressions with mean population CT_{min} and CT_{max} as the dependent variables against the population means for rate of temperature change, initial experimental temperature, and body mass (Supp. Table 3_2). We conducted separate analyses for each pair of dependent and independent variables, and each regression was weighted by the variance in CT_{min} or CT_{max}. Because none of these models were statistically significant (Supp. Table 3_3), we used raw CT_{min} and CT_{max} values in subsequent analyses.

We compared physiological traits to thermal habitat (3 PC variables, see Results) using population means and independent contrasts. We calculated standardized independent contrasts (scaled by the expected variance) for the weighted species means of each physiological trait (CT_{min}, T_b, and CT_{max}) and each of the thermal habitat variables (PC I, PC II, PC III) using the *pic* function in the APE package (Felsenstein 1985; Paradis 2006) in R (R Development Core Team 2012). Although they are not properties of the organisms, environmental traits may reflect underlying ecological traits

(Garland et al. 1992; Oufiero et al. 2011). We used the time-calibrated, majority rule consensus tree of Mahler et al. (2010), with the topology generated using Bayesian maximum clade credibility (Drummond and Rambaut 2007). This consensus tree is the most comprehensive phylogeny of anoles to date and contains 187 of ~375 recognized species of anoles (all but 19 species of Caribbean anoles), including all the species used in this study (Fig. 3_1). We compared the contrasts for physiological traits with the contrasts for thermal environment through regression through the origin using the *lmorigin* function in APE (Paradis 2006).

Measuring rates of physiological evolution

To ensure comparability among traits, we used the fitContinuous function in the GEIGER package (Harmon et al. 2008) in R to fit three different models of evolution to each physiological trait. These models were: (1) Brownian Motion (BM), a random walk; (2) Ornstein-Uhlenbeck (OU), a random walk in which characters tend to return to a single optimum; and (3) Early Burst (EB), in which the overall rate of evolution exponentially slows through time (Hansen 1997; Blomberg et al. 2003; Butler and King 2004; Freckleton and Harvey 2006). We calculated the Akaike Information Criterion corrected for small sample size (AICc; Sugihara 1978) for each model and compared the fits by examining the Akaike weights (Burnham and Anderson 2002).

We used Adams' (2013) method to evaluate whether the rate of evolutionary change varied among physiological traits. This method compares a model that allows rates to vary among traits to one where the rates are constrained to be equal using a likelihood ratio test and AICc. To account for intraspecific measurement error, we incorporated the standard error of the mean in our estimation of rates of evolution. We used the APE library (Paradis et al. 2004; Paradis 2012) and new code supplied by Adams (2013) in R.

Cold acclimation trials

Because of the strong differences in CT_{min} we discovered among populations (see Results), we conducted an experiment to assess how short-term acclimation influences variation in this trait. In June 2013, we collected adult male lizards from two populations from our initial 2011 sampling sites – *Anolis armouri* (Loma de Toro, elevation = 2318 m; n = 18) and *A. cybotes* (Los Patos, elevation = 45 m; n = 19). Kolbe et al. (2012) found that a two-week acclimation at 22.5°C was sufficient to elicit a strong plastic response in CT_{min} in a lowland population of *A. cristatellus* from Puerto Rico without inducing severe thermal stress. We maintained lizards at 19.4°C (range = 17.4°C – 21.9°C) for three weeks and measured CT_{min} following the procedure described above.

Night-time environmental temperature measurement

We measured night-time operative temperature (T_e) in two of our warmest and coldest localities, Los Patos (45 m) and Loma de Toro (2318 m). T_e refers to an organism's equilibrium temperature in the absence of metabolic heating or evaporative cooling (*sensu* Bakken 1992), which we estimated using replicas made of electroformed copper, which mimic the thermal properties of a lizard (e.g., color, shape, size) save for its ability to physiologically regulate its temperature (Bakken and Gates 1975). We constructed 40 copper models shaped using a mold of A. cybotes with an iButton® data logger (DS1921K Maxim) embedded inside – this new generation of copper models permits automated temperature recording (for details of their construction, see Bakken and Angilletta [2013]). Methods for calibrating the copper models are given in the Supplemental Materials (Supp. Mat. 3_1). We deployed 40 models (12 on trees, 11 on rocks, and 11 under rocks) in Los Patos (June 5 – 7, 2013) and 39 models (11 each on trees, on rocks, and under rocks) in Loma de Toro (June 14 – 16, 2013), and the devices automatically recorded T_e every ten minutes. We randomly selected perches, orientation, and height for model placement on trees following Hertz (1992). We recorded sleep site selection for lizards at each of these localities during the experimental period.

Results

Thermal habitat varies markedly across Hispaniola

For this study we visited 15 localities that spanned more that 2400 meters in altitude and a variety of habitats ranging from lowland scrub to montane pine forests. Not surprisingly, sites varied considerably in temperature (Fig. 3_2). In the principal component analysis of the WorldClim thermal variables, we recovered three axes with eigenvalues greater than 1 that together explained 99.6% of the variation in the thermal data (Supp. Tables 3_4 and 3_5). PC I, hereafter termed 'Thermal PC I', explained 73.2% of the variation and loaded highly for mean annual temperature, mean temperatures of the wettest and driest quarters, maximum temperature of the warmest month, and minimum temperature of the coldest month. PC II (14.2% variation), hereafter the 'Range PC II', loaded highly for daily and annual temperature ranges, whereas PC III (12.2% variation explained), or the 'Seasonality PC III' axis, loaded with variables related to seasonality.

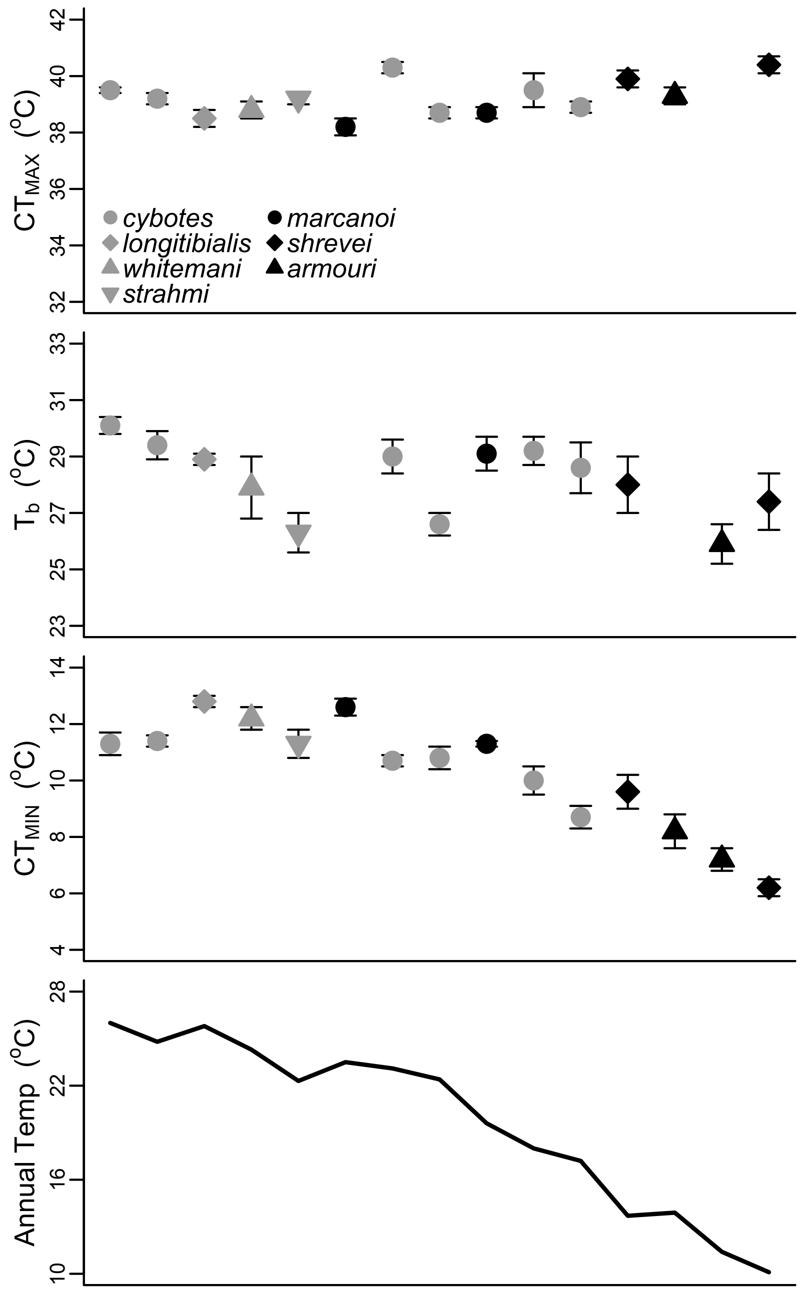

Figure 3_2. From top to bottom, population means (± 1 s.e.m.) are given for thermal maximum, CT_{max}, body temperature (T_b), and critical thermal minimum (CT_{min}). The mean annual temperature for each locality is provided in the bottom panel. The x-axis denotes elevation rank for each population. Species are denoted in different colors and shapes.

Analysis of thermoregulation and physiology

In approximately 164 hours of field observations, we collected T_b from 435 adult male lizards and basking site choice data from 381 lizards. The extent of basking diverged greatly under sunny conditions (logistic interaction term; $\chi^2 = 4.07$, df = 1, $p = 0.044$); lizards at higher elevation were more likely to bask in the sun when it is available, whereas those at lower elevation sought shade. T_b did not vary significantly with any of the thermal habitat PC variables. Tolerance to cold (CT_{min}) was positively correlated with Thermal PC I ($r = 0.934$, $p < 0.001$), whereas CT_{max} did not significantly correlate with any of the PC variables (Table 3_2). All significant correlations remained so after phylogenetic phylogenetic correction (Table 3_2).

Evolutionary analyses of physiology

The Brownian motion (BM) model was the most strongly supported for all three allowing for a comparison of evolutionary rates among traits. Likelihood ratio tests indicated that, overall, the three physiological traits evolved at different rates, although the differences were just above the significance threshold ($p = 0.06$) when intraspecific measurement error was taken into account (Table 3_3). Pairwise comparisons showed that rates of evolution for CT_{min} were significantly higher than for CT_{max}, even when intraspecific measurement error was considered. However, differences in rates of evolution between T_b and other traits were not significant in the analysis incorporating intraspecific variation (Table 3_3), either because rates do not actually differ, or because high variance in T_b obscures differences in rates of evolution.

Table 3_2. Results from linear regressions assessing the relationship between physiological traits (critical thermal minimum, CT_{min}; mean body temperature, T_b; and critical thermal maximum, CT_{max}) and thermal environment (PC I / mean annual temperature, PC II / temperature range, and PC III / temperature seasonality) using population means (left) and independent contrasts of species means (right). Correlations among contrasts were measured using the *cor.table* function in picante in R.

	Populations			**Contrasts**		
	reg. coeff.	Pearson's *r*	*p*	reg. coeff.	Pearson's *r*	*p*
CT_{min}						
PC I	1.87	0.934	<0.001	1.56	0.973	0.001
PC II	-0.20	0.022	0.755	0.05	0.032	0.951
PC III	0.46	0.140	0.503	0.38	0.132	0.790
T_b						
PC I	0.52	0.363	0.142	0.78	0.637	0.143
PC II	-0.35	-0.296	0.341	-0.48	-0.421	0.460
PC III	0.68	0.479	0.054	-0.73	-0.381	0.464
CT_{max}						
PC I	-0.29	-0.618	0.110	-0.24	0.186	0.204
PC II	-0.16	-0.228	0.300	0.13	0.881	0.672
PC III	0.13	0.193	0.524	0.03	0.865	0.957

Table 3_3. Comparison of evolutionary rates for CT_{min}, T_b, and CT_{max}. Top: The full analysis of evolutionary rates (σ^2) incorporating covariation among all three traits. One test accounted for intraspecific measurement error (corrected), whereas the other did not (uncorrected). AIC_C scores for a model that allows rates to vary (observed) among traits and a model that constrains rates of evolution to be equal among traits are given (constrained), and likelihood ratio test results are also given. Bottom: Likelihood ratio tests for pairwise comparisons of evolutionary rates among traits. As above, the results for models that incorporate intraspecific measurement error (corrected) and for models that do not (uncorrected) are presented.

Trait		**Uncorrected**	**Corrected**
full analysis			
CT_{min}	10.60	AIC_C (OBS) = 167.3	AIC_C (OBS) = 171.7
T_b	6.36	AIC_C (CONS) = 175.8	AIC_C (CONS) = 173.2
CT_{max}	0.78	$LRT_{d.f. = 2} = 12.56$, $p = 0.002$	$LRT_{d.f. = 2} = 5.57$, $p = 0.06$
Comparison		Uncorrected $LRT_{d.f.=1}$; p	Corrected $LRT_{d.f.=1}$; p
Pairwise analysis			
CT_{min} vs. CT_{max}		12.40; <0.001	7.85; 0.005
CT_{min} vs. T_b		0.87; 0.350	0.32; 0.574
CT_{max} vs. T_b		6.86; 0.009	-4.70; 1.00

Cold acclimation experiment

Mean CT_{min} was not significantly different between the population of wild-measured *A. cybotes* (mean = 11.3°C) and cold-acclimated *A. cybotes* (mean = 10.6°C) (unpaired *t*-test: $t = 1.53$, $p = 0.136$). Mean CT_{min} remained the same (7.2°C) between wild-measured and cold-acclimated *A. armouri*. CT_{min} was significantly higher in *A. cybotes* than in *A. armouri* in both the wild-measured (unpaired *t*-test: $t = 7.72$, $p < 0.001$) and cold-acclimated treatments ($t = 9.28$, $p < 0.001$).

Night-time temperature experiment

Night-time operative temperatures (T_e) showed marked differences between high and low elevation (Fig. 3_3). In Los Patos (low elevation), T_e ranged from 24.6 – 29.8°C, whereas in Loma de Toro T_e (high elevation), it ranged from 10.9 – 18.1°C. T_e was somewhat higher (though not always) under rocks than on top of rocks or on trees (Fig. 3_3). All lizards in Los Patos were observed sleeping on vegetation (43/43 observations), whereas lizards from Loma de Toro were observed sleeping on vegetation (14/30) and underneath rocks (16/30) in roughly equal numbers (test for differences in site selection among populations: $\chi^2 = 26.3$, df = 1, $p < 0.001$).

Discussion

Thermoregulatory behavior influences the rate of physiological evolution

The question of how behaviour mediates physiological evolution dates back to the middle of the last century (Cowles and Bogert 1944; Bogert 1949) and has received

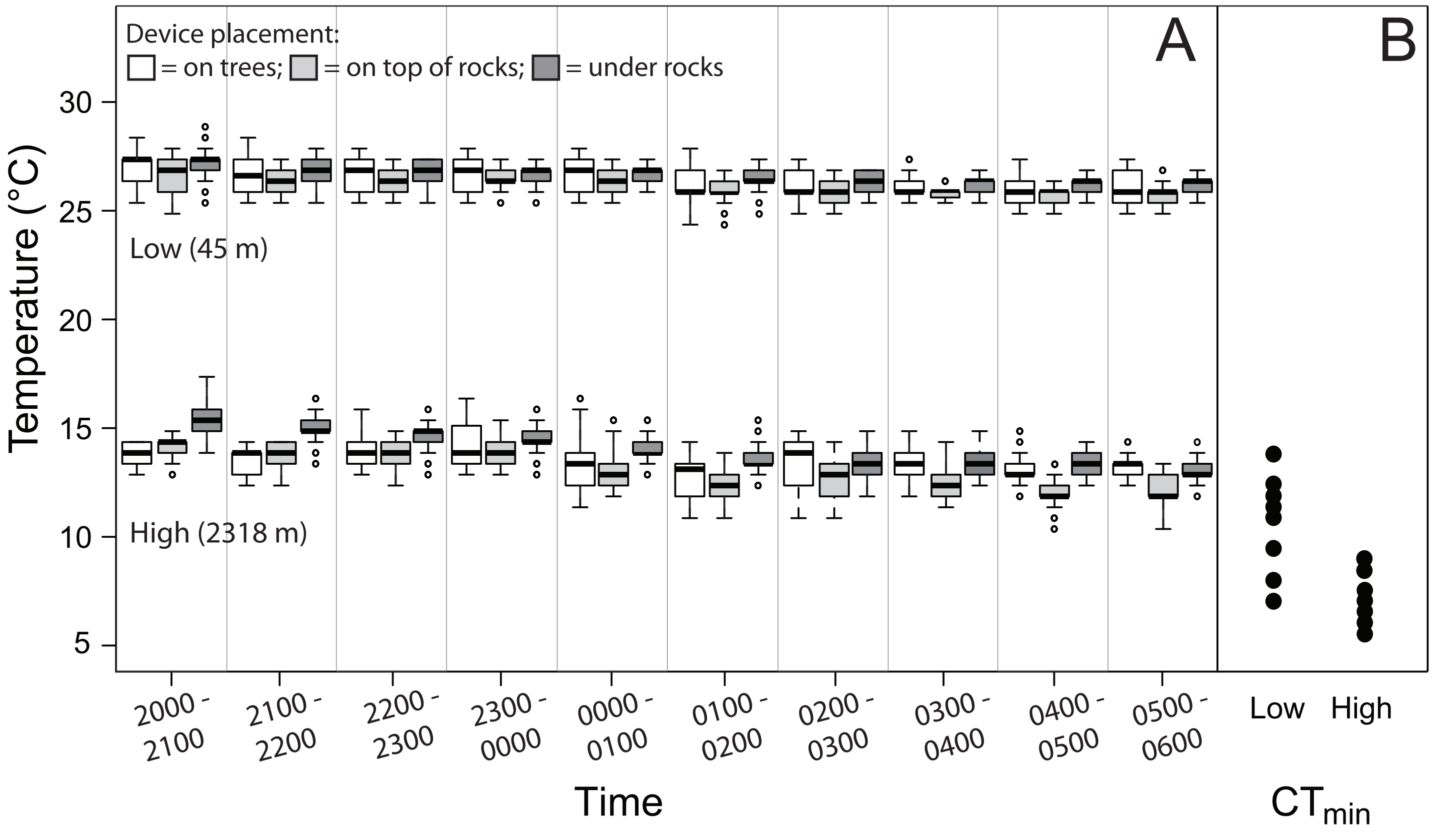

Figure 3_3. (A) Box plots showing the variation in operative temperatures during 1 h time blocks. Each time block summarizes temperatures collected over three consecutive nights in June 2013 at low elevation (45 m) and high elevation (2318 m). Color denotes perch type as follows: on trees, white; on rocks (light gray); and under rocks (dark grey). (B) CT_{min} measured in individuals of *A. cybotes* (left) and *A. armouri* (right) from the same localities in part (A) are given.

renewed interest in the light of concern about how ectothermic organisms can respond to climate warming (Huey et al. 2009; Kearney et al. 2009). We studied a clade of lizard whose species vary markedly in thermal environment from hot semi-299 deserts to cold montane environments. Despite occurring in environments that differ by as much as 15°C in mean annual temperature, field body temperature and heat tolerance were remarkably similar among populations, indicating that behavioral thermoregulation can be extraordinarily effective in limiting exposure to excessively suboptimal temperatures (i.e., 'Bogert effect'; Huey et al. 2003). Our behavioral analysis demonstrates that lizards were more likely to retreat to the shade under sunny conditions, whereas upland lizards were more likely to bask when the sun was out, a result in agreement with previous work on three of these species (Hertz and Huey 1981). Our results are particularly striking given that other anole species exhibit markedly different body temperatures, even when they occur in sympatry (Ruibal 1961; Rand 1964; van Berkum 1986; Hertz et al. 2013, but see Huey and Webster 1976; Gunderson and Leal 2012).

Given the ability of the cybotoids to thermoregulate to approximately the same temperature throughout its range, it is not surprising that CT_{max} also shows very little interspecific variation. However, these lizards have a much more limited ability to thermoregulate at night, particularly at high elevation, where operative temperatures measured on all types of sleep sites were so low that they would incapacitate 80% of lowland lizards (Fig. 3_3; Supp. Fig. 3_1). In the absence of thermal refuges, populations have no option but to adapt physiologically. We found that none of the lizards from high elevation experienced night-time temperatures lower than their CT_{min} (Fig. 3_3).

An alternative explanation for this finding is that differences in CT_{min} represent non-genetic effects of living in different environments. Previous studies suggest that adaptive plasticity is unlikely to account for physiological differences among populations (Janzen 1967): our data support this view, as cold tolerance exhibits little acclimatization, even less so than in other anoles (Kolbe et al. 2012), which suggests that there is probably a genetic basis for the observed variation in CT_{min}.

The inability of thermoregulation to buffer selection on physiology during the night is an explanation for the fast rate of CT_{min} evolution observed in this clade (Table 3_3; Huey et al. 2003). The relative stasis in CT_{max} documented here aligns with results from recent meta-analyses showing that there is less variation in heat tolerance than in cold tolerance in several ectotherm clades (Sunday et al. 2010; Araújo et al. 2013). In short, behavioral thermoregulation allows cybotoid species to maintain similarly warm body temperatures during the day, but not during the night, forcing species in montane environments on Hispaniola to adapt to lower temperatures.

What limits heat tolerance evolution?

Behavioral thermoregulation can help explain why CT_{max} is less variable than CT_{min} in the cybotoids, but not why the response to different environmental conditions involved behavioral, rather than physiological, change. Given that time spent thermoregulating imposes a cost with regards to other activities, such as foraging, predator avoidance, and reproduction (e.g., Huey 1974; Grant and Dunham 1988; Adolph and Porter 1993), it is unclear why selection should favor the maintenance of high body temperatures in montane habitats instead of physiological adaptation to lower temperatures. One possibility is that behavioral modifications are easier to evolve than changes in physiological tolerances (Mayr 1963; West-Eberhard 2003). Given that a myriad of physiological processes (e.g., locomotion, digestion, and growth) are sensitive to temperature, the evolution of physiological tolerances may necessitate the concerted evolution of many genes (discussed in Angilletta 2009, Huey 2009). By contrast, shifts in basking frequency change seasonally within populations, and so behavioral shifts at different elevations may not require substantial evolutionary change. Moreover, even if evolutionary shifts in behavior are required, such changes may require fewer genetic changes than shifts in physiology (Blomberg et al. 2003).

This 'evolution along lines of least genetic resistance' (*sensu* Schluter 1996) explanation suggests that there is no inherent advantage to warmer temperatures, but an alternative explanation for the lack of evolutionary variability in CT_{max} revolves around the fitness benefits of high temperatures. Specifically, selection may favor the maintenance of high body temperatures in cold environments because rates of biochemical reactions increase with optimal temperature (Bennett 1987; Huey and Kingsolver 1989; Huey 2009). Indeed, warm-adapted ectotherms generally experience higher levels of physiological performance than cold-adapted organisms (Savage et al. 2004; Angilletta et al. 2010). However, if this 'hotter is better' hypothesis is true, it still fails to address why low elevation populations have not evolved even higher heat tolerances.

Impacts of climate change

Climate warming will probably have different effects on cybotoids from lowland and upland habitats. Warming temperatures threaten to make current ranges inhospitable for many cool-adapted montane ectotherms, which may force their ranges upward (Parmesan 2006; Chen et al. 2011). By contrast, it is likely that upland cybotoids will benefit, at least in the short term, from climate warming. As the climate warms, environmental temperatures will more often approximate lizards' preferred temperatures, and thus the time lizards need to spend thermoregulating should decrease and the number of hours available for other activities should increase. By contrast, higher temperatures may allow species from lower elevations to migrate upwards, potentially leading to negative interspecific interactions (Huey et al. 2009).

The challenge facing lowland cybotoids will be to avoid stressfully hot temperatures as habitats continue to warm. Many tropical lizards, particularly those near sea level, are already frequently experiencing temperatures exceeding their preferred ranges (Huey et al. 2009; Sinervo et al. 2010). As warming continues, lizards in such lowland populations will eventually be unable to maintain temperatures within their preferred range for long enough periods to survive. At that point, lowland populations can only avoid local extinction by shifting their physiology to adapt to these higher environmental temperatures.

Evolutionary stasis in CT_{max} may suggest a limited ability to evolve and, thus, a heightened vulnerability to environmental warming. Some studies on *Drosophila* support the idea that heat tolerance evolution is genetically constrained, as the amount of genetic diversity for heat tolerance is limited compared with that for cold tolerance (Hoffmann et al. 2003, 2012; Ragland and Kingsolver 2008). The observation that CT_{min} evolves readily in cybotoids and in other ectotherms (Barrett et al 2010; Leal and Gunderson 2012) would tend to support this hypothesis. Nonetheless, it is hard to construe why diverse physiological systems would be constrained from evolving upper, but not lower tolerances. In fact, experiments on *Drosophila* (Gilchrist and Huey 1999) and salmon (Donaldson and Olson 1957) have demonstrated that heat tolerance can increase in response to selection, although there appears to be an upper ceiling on how high heat tolerance can evolve (Hamilton 1973); no similar experiments have been conducted on anoles. Moreover, although cybotoid anoles show relatively little variation in heat sensitivity, some other anole clades have diversified extensively while adapting to different thermal environments (Hertz et al. 2013). Looking more broadly, other lizard species possess heat toelrances that approach 50°C (reviewed in Sunday et al. 2010; Araújo et al. 2013), suggesting that if genetic constraints exist in lizards, they are phylogenetically localized. Finding an explanation for variation among clades in physiological diversity could aid in assessing ectotherms' vulnerability to climate warming, but it is a challenge that will require integration of physiological, behavioral, and evolutionary approaches.

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Chapter 4

Untangling intra- and interspecific effects on body size clines reveals divergent processes structuring convergent patterns in *Anolis* lizards.

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Abstract

Bergmann's rule – the tendency for body size to increase in colder environments – remains controversial today, despite 150 years of research. Considerable debate has revolved around whether the rule applies within or among species. However, this debate has generally not considered that clade-level relationships are caused by both intra- and interspecific effects. In this paper, we implement a novel approach that allows for the separation of intra- and interspecific components of trait-environment relationships. We apply this approach to body size clines in two Caribbean clades of *Anolis* lizards and discover that their similar body size gradients are constructed in very different ways. We find inverse Bergmann's clines – high elevation lizards are smaller bodied – for both the *cybotes* clade on Hispaniola and the *sagrei* clade on Cuba. However, on Hispaniola, the inverse cline is driven by interspecific differences whereas intraspecific variation is responsible for the inverse cline on Cuba. Our results suggest that similar body size clines can be constructed through differing evolutionary and ecological processes, namely through local adaptation or phenotypic plasticity (intraspecific clines), and/or size-ordered spatial sorting (interspecific clines). We propose that our approach can help integrate a divided research program by focusing on how the combined effects of intra- and interspecific processes can enhance or erode clade-level relationships at large biogeographic scales.

Introduction

Large-scale patterns in trait variation that we see today are a major source of phenotypic diversity (Ackerly and Cornwell, 2007; Blackburn et al. 1999; Lepš et al. 2011; Mayr 1956; Meiri 2011). Spatial variation in body size is one such pervasive pattern. In particular, body size is known to vary across thermal environments, such that it may increase (Bergmann's cline), decrease (inverse Bergmann's cline), or vary little with elevation and/or latitude (Ashton and Feldman 2003; Pincheira-Donoso et al. 2008). Bergmann's Rule (*sensu stricto*) was initially proposed to explain the tendency for endotherms in colder climates to be larger bodied. The pattern was described as an interspecific cline manifested in homeothermic animals (Bergmann 1847; James 1970); the proposed mechanism underlying this pattern was the conservation of heat through a smaller surface area-to-volume ratio in larger bodied animals. More recently, however, the rule (*sensu lato*) has been extended to include intraspecific size clines, and ectothermic organisms, especially squamates (Rensch 1938; Ashton and Feldman 2003; de Queiroz and Ashton 2004; Olalla-Tárraga et al. 2006) and insects (Chown and Gaston 2010; Shelomi 2012). As empirical studies accumulated, new mechanistic interpreations underlying Bergmann's cline emerged. These interpretations involve environmental factors correlated with temperature or differences in thermoregulatory ability (Meiri 2011, Olalla-Tárraga 2011).

Despite various empirical and theoretical developments, it remains challenging to bridge studies conducted at different phylogenetic scales. Much debate surrounding Bergmann's Rule has focused on whether it should occur within or among species (Mayr 1956; Blackburn et al. 1999; Meiri 2011), largely considering intra- and interspecific body size clines separately. However, at the clade level, clines of body size (or any other trait) reflect intra- and inter-specific variation. Within species, body size – environment relationships, like other trait-environment correlations, can arise due to local adaptation and/or phenotypic plasticity (Phillimore et al. 2010, 2012). However, differences in mean body size among species (beyond the effects of local adaptation and plasticity) may also contribute to body size clines due to evolutionary divergence and size-structured species turnover, such that larger species replace smaller ones along an environmental gradient (Blackburn et al. 1999; Meiri and Thomas 2007). Lastly, species-specific differences in local adaptation and plasticity may alter these effects. Thus, factors operating both within and among species may enhance or degrade clade-level clinal relationships.

In this study, we take an integrative approach by simultaneously considering intra- and interspecific contributions to body size clines. At the interspecific level, we focus on cross-taxon comparisons, rather than assemblage-based approaches (*sensu* Olalla-Tárraga 2011). Specifically, we formulate and test eight competing hypotheses (Fig. 4_1; Table 4_1) for how intra- and interspecific factors can interact to construct body size clines along elevation gradients. Body size clines may be solely explained by local adaptation and plasticity, with no additional interspecific contributions, leading to a single overall cline (H1). Alternatively, either interspecific divergence or local adaptation and plasticity may be the only source of variation, resulting in purely interspecific (H2) or intraspecific clines (H3). When body size clines are due to a mixture of intra- and interspecific effects, they may be concordant (H4) or discordant (H5). Additionally, there may be varying intraspecific clines, either with an interspecific cline (H6), or without (H7). Finally, there may be no intra- or interspecific clines whatsoever (H8).

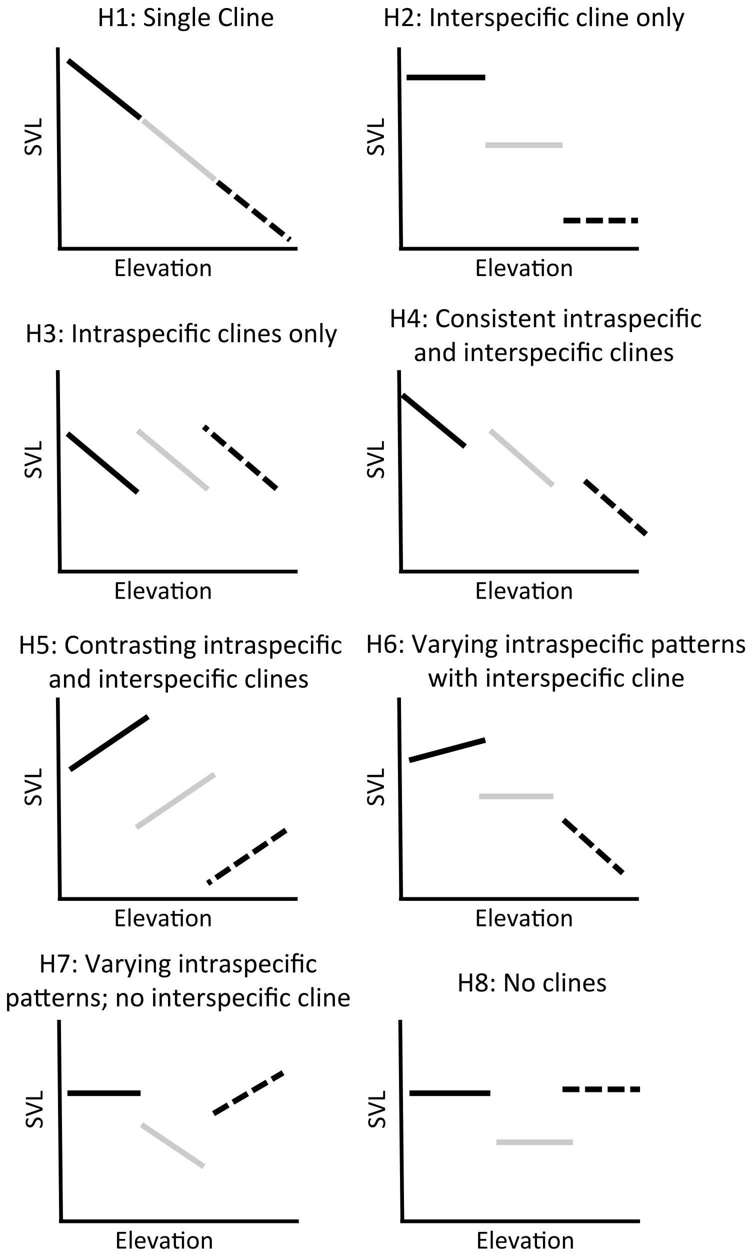

Figure 4_1. Hypotheses for the construction of body size clines along elevational gradients from intra- and interspecific clines. In each plot, three hypothetical species (solid, grey, dashed) are shown. Inverse Bergmann's clines are depicted (decreasing size with increasing elevation), as these are often observed in squamates, but the hypotheses apply identically to positive Bergmann's clines.

Table 4_1. Predictions of the eight hypotheses for the construction of body size clines along elevational gradients. The linear models used to test each prediction are indicated in parentheses. Model 1 is a linear regression of SVL on elevation, pooling all species. Model 2 is a one-way ANOVA to test for mean body size differences among species. Model 3 in an ANCOVA that constrains SVL-elevation slopes to be identical but allows intercepts to vary. Model 4 is an ANCOVA with an interaction term that allows both slopes and intercepts to vary. Model 5 is the regression of species' mean SVL on species' mean elevation.

Hypothesis	Predictions
H1	1. Significant SVL – elevation slope (Model 1)
	2. No difference in intercepts after accounting for elevation (Model 3 vs. Model 1)
	3. No slope differences among species (Model 4 vs. Model 3)
H2	1. Significant difference in species means (Model 2)
	2. No significant individual SVL – elevation slopes (individual species regressions)
	3. Significant relationship between mean SVL and mean elevation (Model 5)
H3	1. No differences in species means (Model 2)
	2. Significant slope after accounting for intercept differences (Model 3 vs. Model 2)
	3. No slope differences among species (Model 4 vs. Model 3)
	4. All individual SVL – elevation slopes significant in the same direction (individual species regressions)
H4	Significant intercept differences after accounting for elevation (Model 3 vs. Model 1)
	2. Significant slope after accounting for intercept differences (Model 3 vs. Model 2)
	3. No slope differences among species (Model 4 vs. Model 3)
	4. All individual SVL – elevation slopes significant in same direction (individual species regressions)
	5. Significant relationship between mean SVL and mean elevation (Model 5)
H5	1. Significant intercept differences after accounting for elevation (Model 3 vs. Model 1)
	2. No slope differences among species (Model 4 vs. Model 3)

Table 4_1 continued.

	3. All individual SVL – elevation slopes significant in same direction (individual species regressions)
	4. Significant relationship between mean SVL and mean elevation
	5. Direction of mean SVL – elevation and individual SVL – elevation slopes differ (individual species regressions and Model 5)
H6	1. Significant difference in species means (Model 2)
	2. Significant relationship between mean SVL and mean elevation (Model 5)
	3. Significant slope differences among species (Model 4 vs. Model 3)
H7	1. Significant slope differences among species (Model 4 vs. Model 3)
	2. No relationship between mean SVL and mean elevation (Model 5)
	3. At least one individual species' SVL – elevation slope is significant (individual species regressions)
H8	1. No relationship between SVL and elevation (Model 1)
	2. No relationship between mean SVL and mean elevation (Model 5)
	3. No significant individual SVL – elevation slopes (individual species regressions)

We test these eight hypotheses using two clades of Caribbean *Anolis* lizards from Cuba and Hispaniola as model systems. Divergence in body size is an important feature of the adaptive radiation of Greater Antillean *Anolis* lizards (Williams 1983; Losos 2009). For this study we focus on body size divergence within two deeply divergent clades from Cuba and Hispaniola. On these islands, lizards are found in environments ranging from scrubby semi-desert to montane pine forests, which pose different selective pressures (Glor et al. 2003; Muñoz et al. 2014a) and provide the opportunity for body size adaptation. By comparing the intra- and interspecific contributions to body size on ecologically convergent clades of *Anolis* lizards in similar thermal environments. both of these islands, we test whether size clines are replicated across independent, but

Methods

Data Collection

We assembled body size data of sixteen *Anolis* species from the *cybotes* (seven species) and the *sagrei* (nine species) clade from Hispaniola and Cuba, respectively. Anoles from both clades are found in nearly all the available habitats on their islands (Henderson and Powell 2009). Further, both clades are similar in that they include a mix of widespread (e.g. *A. cybotes* on Hispaniola and *A. sagrei* on Cuba) and range-restricted species (e.g., *A. shrevei* on Hispaniola and *A. rubribarbus* on Cuba) (Glor et al. 2003; Knouft et al. 2006), suggesting that differences in species delimitation between clades is unlikely to bias our results. We measured body size from ethanol-preserved specimens in the herpetological collection at the Museum of Comparative Zoology at Harvard University, ensuring a minimum of five individuals from at least two localities for each species (Table 4_2). Sample sizes were not significantly different between islands (Wilcoxon rank sum statistic = 28.5, n= 7 and 9, $p > 0.75$). We focused on adult male lizards for this study because females are less well represented in museum collections. We identified males by the presence of enlarged post-anal scales and a visibly enlarged throat fan (dewlap), and measured snout-vent length (SVL) using digital callipers to the nearest 0.01 mm. We recorded SVL as the distance between the tip of the snout and the opening of the cloaca. One person (J. E. W.) performed all measurements, which were taken twice per lizard to assure that deviation between the measurements was less than 1%; if it was not, a third measurement was taken. We retained the average of these measurements as the body size for each lizard. To control for body size evolution caused by factors unrelated to temperature that can occur on small islands, such as niche expansion and ecological release (Schoener 1969; Lister 1976a, b), we restricted our analyses to mainland Cuba and Hispaniola. Body size and elevation data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.q39h2 (Muñoz et al. 2014b).

Table 4_2. Summary information for the sixteen *Anolis* species examined in this study. Species from Hispaniola belong to the *cybotes* clade whereas species from Cuba belong to the *sagrei* clade. Unique x, y refers to the number of unique localities for each species.

Species	N	Unique x,y	mean (s.d.)	Range	mean (s.d.)	Range
			Hispaniola			
armouri	29	6	1462 (482)	887-2122	57 (5.1)	45.9-65.5
cybotes	300	75	350 (437)	0-2100	58.8 (7)	39.8-73.5
longitibialis	26	6	97 (76)	6-161	68.1 (4)	57.7-72.7
marcanoi	40	22	374 (237)	8-1198	55.1 (4.3)	46.4-63.7
shrevei	17	4	2312 (165)	1871-2381	51.2 (3.3)	46.2-56.9
strahmi	16	2	87 (118)	58-528	65.3 (7.4)	50.8-77.9
whitemani	7	4	467 (271)	13-686	56.3 (5)	51.2-65.6
			Cuba			
ahli	9	4	600 (129)	451-774	49.8 (5.2)	44.1-59.1
allogus	96	17	199(299)	6-1136	52.6 (5.6)	37.4-63.4
homolechis	268	39	167 (280)	3-1136	50.1 (4.5)	36.9-66.2
jubar	45	8	55 (42)	4-119	49.6 (3.5)	37.5-57.2
mestrei	13	5	82 (38)	9-189	47.2 (6.4)	37.2-55.1
ophiolepis	13	7	105 (35)	37-151	34.7 (2.3)	31.8-40.4
quadriocellifer	5	3	14 (4)	10-18	45 (3.7)	40.5-49.9
rubribarbus	16	4	24 (12)	10-38	55 (4.5)	46.2-60.6
sagrei	206	39	96 (180)	2-1136	50.1 (4.4)	39.5-61.6

Regression Models and Hypothesis Testing

To test hypotheses of how body size varies with elevation within and among species (H1–H8; Fig. 4_1), we fit a series of linear models to the *cybotes* (Hispaniola) and *sagrei* (Cuba) clades. Figure 4_1 depicts how inverse clines, commonly observed in squamates

(Ashton and Feldman 2003), may arise through the combination of intraspecific and interspecific effects. We used these models to evaluate our eight hypotheses by testing whether incorporating slope and or intercept differences explained significantly more variation than simpler models, using analysis of variance on marginal sums of squares. Testing the hypotheses requires assessing results from multiple models; specific predictions and how they were tested using the various regression models are described in Table 4_1.

Model 1 was a simple linear regression of SVL on elevation, pooling all species within a clade; this assumed that all species shared a common intercept and slope (e.g. H1). Model 2 was a one-way ANOVA that tested for differences in mean SVL among species, assuming no intraspecific relationships between SVL and elevation (e.g. H2 and H8). Model 3 was an ANCOVA that allowed intercepts of the SVL – elevation relationship to vary among species, but constrained slopes to be equal; this model also allowed us to assess whether intra- and interspecific patterns worked in concert or in opposition (e.g. H3, H4, and H5). Finally, Model 4 was an ANCOVA with an interaction that allowed both slopes and intercepts to vary, which allowed us to assess whether intra- and interspecific effects varied in strength and direction (e.g. H6, H7). Each of our hypotheses makes a number of predictions that can be tested by evaluating these models. For example, Hypothesis 1 (Fig. 4_1) predicts a significant relationship between SVL and elevation (Model 1), no difference in species' intercepts after accounting for elevation (testing Model 3 versus Model 1), and no difference in slopes between species (testing Model 4 versus Model 3).

In all models elevation was log-transformed after adding 0.5, to reduce right skew. We accounted for vastly different sample sizes among species by weighting observations by the inverse of their species' sample size. This weighting ensured the relationships were not dominated by a few, prevalent species (e.g. *A. sagrei* on Cuba and *A. cybotes* on Hispaniola). Given that the interaction term in Model 4 evaluates whether the slope of the SVL – elevation relationship varies among species (H6 and H7), but not whether individual species' slopes differ from zero (H2 and H8), we also regressed SVL on elevation individually for each species.

Model 2 tests whether species' means differ, but not whether those differences are related to elevation (e.g., H8 versus H2). To test this we calculated the mean SVL and mean elevation of each species. Using these mean values, we fit another regression model (Model 5) with species' mean SVL as the response variable and mean elevation as the predictor. Thus, for the *cybotes* clade (Hispaniola) n=7 and for the *sagrei* clade (Cuba) n=9. Because a species' mean elevation and body size may be strongly affected by sampling variation along its elevational range, we also fit Model 5 using species' SVL and elevational midpoints rather than means. To account for relatedness among species, we used Mahler et al.'s (2010) maximum clade credibility tree to fit Model 5 for each clade with phylogenetic generalized least squares (PGLS) using the *gls* function in the nlme package (Pinheiro et al. 2013) in R v.2.15.3 (R Development Core Team 2013), and assuming a Brownian motion (BM) model for body size evolution. We chose BM because it fit the data better than an Ornstein-Uhlenbeck model in all cases ($\Delta AIC_c > 4.8$ for all comparisons).

Spatial Autocorrelation

Specimens obtained from identical or nearby localities are not expected to be independent, due to dispersal, gene flow, relatedness or other, unmeasured environmental variables. Following Dormann et al. (2007), we accounted for spatial autocorrelation by fitting generalized least squares models using the *gls* function in the nlme package (Pinheiro et al. 2013) in R v.2.15.3. We fit each model using three different spatial correlation structures: exponential, Gaussian, and spherical, and selected the one with the lowest AIC_c for further significance testing as above. Many specimens were collected at identical localities. Thus, to avoid distances of zero separating spatial neighbors, we added a random jitter to X and Y coordinates. These were randomly drawn from a normal distribution with a mean of zero and a standard deviation of 0.1 m. We repeated jittering 100 times, refitting GLS models each time. Results from GLS models were nearly identical to non-spatial models and led to the same conclusions; thus, spatial autocorrelation cannot account for the observed results. We present only non-spatial results below and GLS results can be found in the Appendix (Supp. Materials 4_1).

Comparing elevational range

The detected relationships between elevation and body size within species may depend on a species' elevation range. However, we found no significant difference in the mean elevational range occupied by Hispaniolan species versus Cuban species (Hispaniola: mean ± s.e. = 904.7 ± 247.8 m; Cuba: mean ± s.e. = 462.8 ± 170.1 m, t = -1.52, d.f. = 14, $p = 0.15$). Furthermore, intraspecific clines may be easier to detect in species with wide elevational ranges. To test for this, we regressed the absolute value of individual species' SVL – elevation slope against elevation range, weighting points by the standard error of the slope estimate. After log-transforming individual species' regression slopes because of highly skewed residuals, and pooling species from both islands, we found a negative relationship between individual absolute SVL – elevation regression slopes and elevation range (Supp. Fig. 4_1; GLM slope ± s.e. = $-1.1\times10^{-3} \pm 4.6\times10^{-4}$, $t = -2.40$, d.f. = 14, $p = 0.006$). Thus, species with narrower ranges actually tended to have steeper body size clines, though not necessarily in a consistent direction. However, as species' elevational ranges did not differ among islands, this is unlikely to underlie any observed differences between the two clades.

Results

Body size – elevation relationships in the cybotes *clade (Hispaniola)*

We found that body size was negatively related to elevation on Hispaniola when data from all species were pooled together (Model 1), indicating an inverse Bergmann's cline (Fig. 4_2; slope ± s.e. = -1.95 ± 0.18, $t = -10.60$, d.f. = 433, $p < 10^{-15}$). Elevation accounted for 21% of the variance in body size (Table 4_3). Mean body sizes differed significantly among species (Model 2; Table 4_3) and accounted for 53% of the total variance. We also found that the intercepts, but not the slopes of the SVL – elevation relationships, varied among species (Model 3), revealing that the negative Bergmann's cline was primarily due to inter- rather than intraspecific differences (Table 4_3; Fig. 4_2). In this model, species' intercepts were significantly different, even after accounting for elevation, whereas the elevation term was not significant after accounting for intercept differences (Table 4_3).

Table 4_3. Analysis of variance of four linear models fit to the *cybotes* clade on Hispaniola and *sagrei* clade on Cuba.

cybotes clade, Hispaniola

Model	Common slope				Intercept differences				Slope differences				Model
	F	d.f.	p	Partial R^2	F	d.f.	P	Partial R^2	F	d.f.	p	Partial R^2	R^2
1	112.3	1,433	$< 1\times10^{-15}$	0.21	-	-	-		-	-	-	-	0.21
2	-	-	-	-	79.1	6, 428	$< 1\times10^{-15}$	0.53	-	-	-	-	0.53
3	1.0	1 427	0.31	1.1×10^{-3}	48.3	6, 427	$< 1\times10^{-15}$	0.32	-	-	-	-	0.53
4	-	-	-		-	-	-	-	1.6	6,421	0.32	0.14	0.54

sagrei clade, Cuba

Model	Common slope				Intercept differences				Slope differences				Model
	F	d.f.	p	Partial R^2	F	d.f.	p	Partial R^2	F	d.f.	p	Partial R^2	R^2
1	14.3	1,669	$< 1\times10^{-3}$	0.021	-	-	-		-	-	-		0.021
2	-	-	-		124.1	8, 662	$< 1\times10^{-15}$	0.60	-	-	-		0.60
3	123.4	1,661	$< 1\times10^{-15}$	0.0074	12.5	8, 661	$< 1\times10^{-3}$	0.59	-	-	-		0.61
4	-	-	-		-	-	-		16.1	8,653	$< 1\times10^{-3}$	0.065	0.67

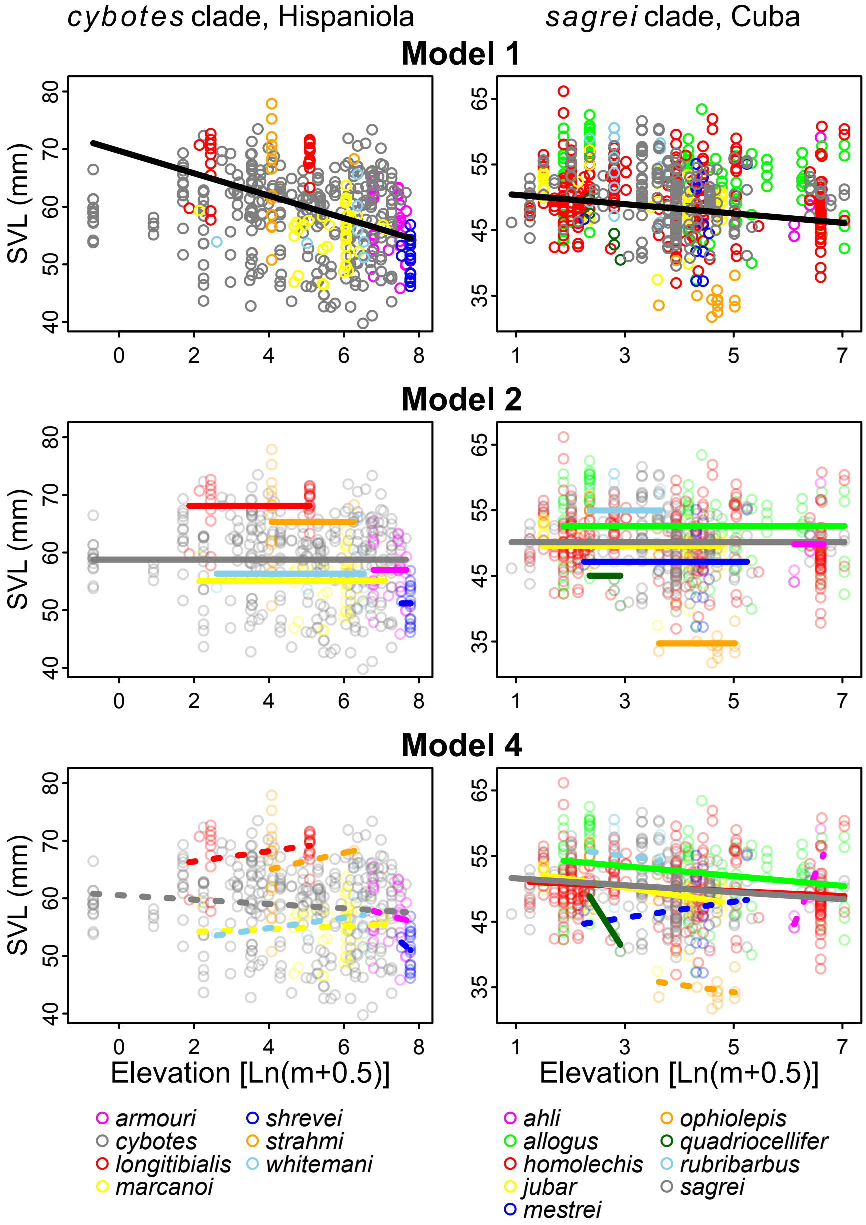

Figure 4_2. Body size (snout-vent length; SVL) – elevation relationships for the *cybotes* clade on Hispaniola and the *sagrei* clade on Cuba. Model 1 fit a single relationship to all species ($p < 0.05$ in both cases). Model 2 constrained slopes at zero and allowed intercepts to vary ($p < 0.05$ in both cases), and Model 4 allowed slopes and intercepts to vary; dashed lines depict slopes that were not significant ($p > 0.05$) using GLS models that

incorporated spatial autocorrelation (Supp. Table 4_1). To ensure best fit lines are visible, points are faded for Models 2 and 4. Model 3 is not shown because the value of the common slope is arbitrary. Results for the *cybotes* clade are consistent with Hypothesis 2, whereas results for the *sagrei* clade are consistent with Hypothesis 7 (Fig. 4_1).

Additionally, the percentage of variance uniquely attributable to intercept differences (32%) was far greater than that uniquely attributable to elevation (0.1%; Table 4_3). Lastly, we found no evidence that the slopes of body size relationships varied among species (Fig. 4_2), as the interaction in Model 4 was not significant (Table 4_3).

The lack of a significant elevation effect in Model 3 suggests that interspecific differences drive the inverse Bergmann's cline in the *cybotes* clade. The inverse relationship between species' mean SVL and mean elevation (Model 5) further corroborates this interpretation (Fig. 4_3; slope ± s.e. = -4.27 ± 0.99, t = -4.32, d.f. = 5, $p = 0.008$). Nearly identical results were obtained when species' midpoints, rather than means were used (not shown), and when PGLS was used to account for relatedness among species ($p < 0.025$ for PGLS slopes). Lastly, individual species' regressions of SVL on elevation revealed that slopes were not significantly different from zero (Table 4_4). The lack of significant intraspecific relationships coupled with the overall inverse body size cline (Model 1) and a negative cline in mean body size (Model 5) are consistent with Hypothesis 2 (Fig. 4_1), in which the clade-level inverse cline is due solely to interspecific effects.

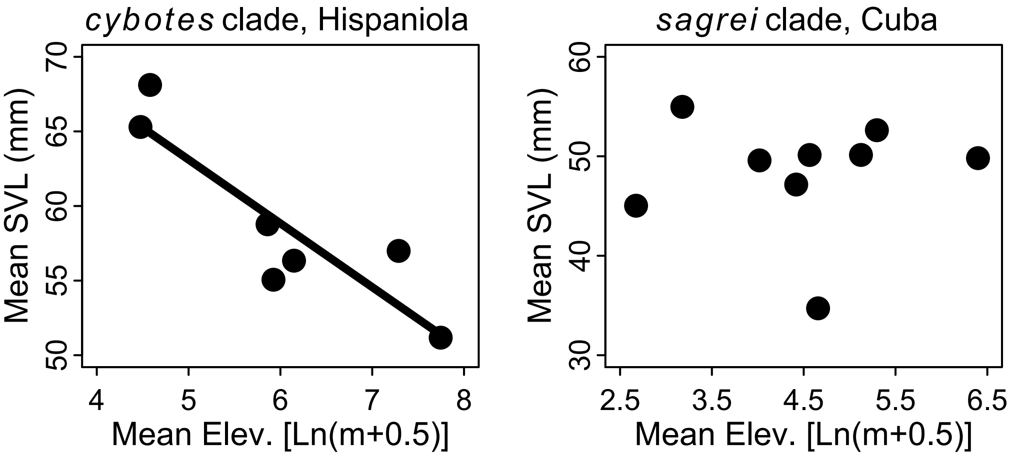

Figure 4_3. Relationship between species' mean body size (snout-vent length; SVL) and the mean elevation of their sampled localities for the *cybotes* clade on Hispaniola ($p <$ 0.01) and *sagrei* clade on Cuba ($p > 0.90$). The relationships were nearly identical when midpoints, rather than means, were used (not shown).

Body size – elevation relationships in the sagrei *clade* (Cuba)

When species identity was ignored (Model 1), we found that SVL decreased with elevation in the *sagrei* clade (Fig. 4_2; slope ± s.e. = -0.71 ± 0.19, t = -3.78, d.f. = 669, $p < 2\times10^{-4}$), but only weakly so, explaining only 2.1% of the variance (Table 4_3). Thus, we detected an overall inverse Bergmann's cline for the *sagrei* clade anoles.

As with the *cybotes* clade anoles, there were significant differences among species' mean body sizes that explained 60% of the variance in SVL (Model 2; Table 4_3). However, in contrast to the results from Hispaniola, we recovered significant intraspecific relationships between body size and elevation in the Cuban *sagrei* clade (Fig. 4_2, Table 4_3). Specifically, the elevation term in Model 3 remained statistically significant after incorporating species differences, though it uniquely explained extremely little variance (Table 4_3). This is in part because the SVL – elevation relationships differed among species (Fig. 4_2; Table 4_3). When the variance explained by the Model 3 was partitioned, we found that the intercept differences and elevation independently explained 59% and 0.7% of the variance, respectively (Table 4_3). Allowing the slopes to vary (Model 4) accounted for an additional 6.5% for a total of 67%, demonstrating that intraspecific variation is relatively slight relative to interspecific differences in SVL in this clade (Table 4_3). However, interspecific differences were not responsible for overall SVL clines, as we found no significant relationship between species' mean SVL and mean elevation (Model 5; Fig. 4_3; slope ± s.e. = 0.21 ± 1.97, t = 0.11, d.f. = 7, $p = 0.92$). These results were nearly identical when midpoints, rather than means, were used (not shown) and remained non-significant when PGLS was used ($p > 0.55$ for both mean and midpoint analysis). Regressions of SVL on elevation for individual species revealed that body size decreased significantly with elevation in five out of nine species (*Anolis allogus, A. homolechis, A. jubar, A. sagrei* and *A. quadriocellifer*) (Fig. 4_3; Table 4_4). These results closely mirrored those from our GLS analysis, with the exception of *A. ahli*. Although *A. ahli*'s body size apparently increased with elevation, this relationship was not significant in our GLS analysis (Supp. Table 4_2). In summary, as with the *cybotes* clade anoles, the *sagrei* clade anoles exhibited an inverse Bergmann's cline. However, unlike in the *cybotes* clade, there was no clear interspecific cline for the *sagrei* clade. Rather, within species, several species showed significant body size – elevation relationships. Given that strength and direction of the size clines differed, our results are most consistent with Hypothesis 7 for the *sagrei* clade on Cuba.

Table 4_4. Body size - elevation relationships for individual species in the *cybotes* clade on Hispaniola and the *sagrei* clade on Cuba. Body size was measured as snout-vent length. Elevation was natural log transformed (after adding 0.5). Significant ($p < 0.05$) models are in bold. These results were closely mirrored in the GLS analysis, save for *A. ahli* (Supp. Table 4_2).

Species	**Slope (s.e).**	**t**	**d.f.**	***p***	**R^2**
***cybotes* clade, Hispaniola**					
armouri	-1.83 (2.69)	-0.68	27	0.50	0.017
cybotes	-0.38 (0.20)	-1.93	29	0.055	0.012
longitibialis	0.89 (0.56)	1.58	24	0.13	0.095
marcanoi	0.24 (0.84)	0.28	38	0.78	0.0021
shrevei	-5.59 (10.69)	-0.52	15	0.61	0.018
strahmi	1.45 (3.59)	0.40	14	0.69	0.011
whitemani	0.90 (1.46)	0.04	5	0.57	0.070
***sagrei* clade, Cuba**					
ahli	**19.75 (5.00)**	**3.95**	**7**	**0.0055**	**0.69**
allogus	**-0.75 (0.33)**	**-2.24**	**94**	**0.028**	**0.051**
homolechis	**-0.37 (0.16)**	**-2.34**	**26**	**0.020**	**0.020**
jubar	**-1.25 (0.39)**	**-3.17**	**43**	**0.0028**	**0.19**
mestrei	1.22 (2.88)	0.42	11	0.68	0.016
ophiolepis	-1.14 (1.65)	-0.69	11	0.50	0.042
quadriocellifer	**-12.98 (2.62)**	**-4.96**	**3**	**0.016**	**0.89**
rubribarbus	-1.03 (2.45)	-0.42	14	0.68	0.013
sagrei	**-0.53 (0.26)**	**-2.03**	**20**	**0.043**	**0.020**

Discussion

The *cybotes* and *sagrei* clades both exhibit an inverse Bergmann's cline; lizards tend to be smaller at high elevations than near sea level. Inverse Bergmann's clines have been previously documented in squamates (Ashton and Feldman 2003; Olalla-Tárraga et al. 2006), though they are by no means ubiquitous (Pincheira-Donoso and Meiri, 2013). Inverse size clines may arise from a number of mechanisms. First, small body sizes at high elevations may be adaptive for thermoregulation. Due to their greater surface area to volume ratio, smaller-bodied individuals warm and cool more rapidly, which may allow them to more precisely thermoregulate in cold montane environments (Ashton and Feldman 2003; Sears and Angilletta 2004).

Indeed, previous work has demonstrated that montane lizards in the Hispaniolan *cybotes* clade thermoregulate during the day at temperatures comparable to their lowland counterparts (Hertz and Huey 1981; Muñoz et al. 2014a). Low thermal inertia may aid this thermoregulatory behavior of smaller, montane lizards. Current evidence suggests that body temperatures and basking tendencies differ considerably among species in the *sagrei* clade (Ruibal 1961; Rodríguez-Schettino et al. 2010; Hertz et al. 2013). Thus, it seems possible that lizard species in the two clades show different relationships between body size and thermoregulatory behavior. However, given that the anoles examined are all relatively small-bodied lizards, it also remains unclear whether the magnitude of size differences among species is sufficient to significantly alter thermal inertia.

Inverse size clines may also reflect differences in life history between low and high elevation lizards. Colder temperatures typically slow growth in ectotherms. This slower growth may produce smaller adult body size if colder temperatures also reduce survival to maturity or reduce fecundity, relative to lizards inhabiting warmer temperatures (Angilletta et al. 2004a,b, but see Sears and Angilletta 2004). Given that high elevation lizards in the *cybotes* clade retain their eggs until a later developmental stage before laying (Huey 1977) and that anoles lay one egg at a time (discussed in Losos

2009), it is possible that smaller body size at maturity may serve to compensate for reduced fecundity in montane species. Alternatively, smaller body size at high elevation may also be non-adaptive and be the result of reduced activity times that limit growth (Sears and Angilletta 2004; Caruso et al. 2014). Finally, individuals are also subject to a range of abiotic and biotic selection pressures beyond temperature, including but not limited to microhabitat availability, competition, predation, disease, and resource availability, all of which may influence body size (Angilletta et al. 2004b, Pincheira-Donoso and Meiri 2013).

Although the precise mechanisms underlying inverse size clines remain unknown, it is clear that they were constructed in different ways on Cuba and Hispaniola. For the Hispaniolan *cybotes* clade interspecific size differences are responsible for the cline, with little or no evidence for intraspecific contributions (H2 in Figure 4_1). Thus, the *cybotes* clade cline reflects the evolutionary divergence of species' body size, coupled with size-ordered species turnover, or the replacement of larger species with smaller ones as elevation increases (Blackburn et al. 1999; Ackerly and Cornwell 2007; Lepš et al. 2011). Alternatively, in the Cuban *sagrei* clade, intraspecific body size clines are responsible for the overall inverse Bergmann's cline, with no evidence for contributions from interspecific body size differences. We suggest that these results are consistent with Hypothesis 7 (Fig. 4_1), as there are varying intraspecific patterns, but no clear interspecific cline. Thus, in this clade, variation in local adaptation and/or phenotypic plasticity likely underlies the cline, rather than evolutionary divergence in body size among species.

The differential role of interspecific and intraspecific processes between the two clades raises many questions, especially because the adaptive radiation of Caribbean anoles is characterized by replicated, convergent evolution in microhabitat use and functional adaptations (Williams 1972, 1983; Losos 2009). One possibility is that differences in the physical geography of Cuba and Hispaniola may underlie the different patterns observed on each island. Hispaniola's highland areas (*sensu* Wollenberg et al. 2013) and their associated climatic gradients, are far more extensive than on Cuba (Supp. Fig. 4_4). Hispaniola harbors 7,940 km^2 of habitat above 1,000 m.a.s.l., whereas Cuba has only 271 km^2 of highland habitat. The greater extent of climatically extreme habitats suggests a greater potential for reduced dispersal and isolation by environment along tropical elevational gradients (*sensu* Janzen 1967) in Hispaniola. Given that speciation depends on an area threshold on oceanic islands (Losos and Schluter 2000; Kisel and Barraclough 2010; Losos and Parent 2010), the same may be true for highland habitat. Hispaniola's extensive highland environments may create ecological isolation that triggers speciation. This speciation may be preceded by or followed by selection for reduced body size. Importantly, the ecological isolation would more likely produce stronger interspecific clines while reducing intraspecific variation. Conversely, climatically extreme habitat is more rare on Cuba. As a result, higher gene flow across elevations may have enabled the broader intraspecific clines to arise here.

The intraspecific clines observed in the Cuban *sagrei* clade varied considerably in strength and direction. Body size is important for multiple dimensions of an organism's biology, including physiology, ecology, and life history. Thus, body size is likely under various types of selection at the same time, which may work in concert or opposition to shape clinal patterns. For example, geographic variation in sexual selection is known to enhance or erode body size clines, particularly in species that exhibit sexual size dimorphism (Storz et al. 2001; Blanckenhorn et al. 2006). In anoles, sexual selection may promote large male size to aid defence of territories and to increase access to mating opportunities (reviewed in Butler et al. 2000, Losos 2009). Thus, variation in the strength and direction of intraspecific clines in male body size may reflect a combined response to sexual and natural selection, possibly to the extent that narrowly restricted species exhibit steeper clines than broadly distributed species, as we observed. Females in the *sagrei* clade exhibit much less variation in body size than males (Henderson and Powell 2009). This point highlights that our findings stem only from males. Although female size is likely correlated with male size, it remains unknown if intraspecific or interspecific variation creates body size-elevation clines on these islands.

In this article we introduced an approach that is widely applicable for parsing out the contributions of intra- and interspecific variation to trait-environment relationships. We found that patterns apparent at the level of clades may be absent or nearly so at the level of species, and vice versa. Thus, similar clinal patterns in body size can be constructed from different ecological and evolutionary processes. Our results demonstrate that restricting analyses of Bergmann's Rule (or other ecogeographical patterns) solely to either the intra- or interspecific level may miss important trait – environment relationships and, consequently, affect inferences of their underlying ecological and evolutionary factors. The analyses we present here show that mechanisms underlying biogeographic patterns should be considered in a framework that explicitly considers how local adaption and trait divergence during speciation can produce similar patterns.

Integrating both intra- and interspecific effects can improve our understanding of how geographic patterns in trait distributions emerge, and may prove useful to explain a wide variety of evolutionary phenomena.

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Chapter 5

Divergence in coloration and ecological speciation in the *Anolis marmoratus* species complex.

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Abstract

Adaptive divergence in coloration is expected to produce reproductive isolation in species that use colorful signals in mate choice and species recognition. Indeed, many adaptive radiations are characterized by differentiation in colorful signals, suggesting that divergent selection acting on coloration may be an important component of speciation. Populations in the *Anolis marmoratus* species complex from the Caribbean island of Guadeloupe display striking divergence in the color and pattern of adult males that occurs over small geographic distances, suggesting strong divergent selection. Here we test the hypothesis that divergence in coloration results in reduced gene flow among populations. We quantify variation in adult male coloration across a habitat gradient between mesic and xeric habitats, use a multilocus coalescent approach to infer historical demographic parameters of divergence, and examine gene flow and population structure using microsatellite variation. We find that color variation evolved without geographic isolation and in the face of gene flow, consistent with strong divergent selection and that both ecological and sexual selection are implicated. However, we find no significant differentiation at microsatellite loci across populations, suggesting little reproductive isolation and high levels of contemporary gene exchange. Strong divergent selection on loci affecting coloration probably maintains clinal phenotypic variation despite high gene flow at neutral loci, supporting the notion of a porous genome in which adaptive portions of the genome remain fixed whereas neutral portions are homogenized by gene flow and recombination. We discuss the impact of these findings for studies of color evolution and ecological speciation.

Introduction

Recent theoretical and empirical support for models of ecological speciation and divergence with gene flow have sparked renewed interest in the role that divergent natural selection and local adaptation plays in the evolution of reproductive isolation (Rice and Hostert 1993; Dieckmann and Doebeli 1999; Gavrilets 2004; Nosil 2008, 2012). Many studies have demonstrated that local adaptation in response to divergent selection across ecological gradients can generate phenotypic diversity in spite of high gene flow (Smith et al. 1997; Schneider et al. 1999; Freedman et al. 2010; Kirschel et al. 2011) and that the role of clinal adaptive variation in ecological speciation is potentially strong (Endler 1977; Rundle and Nosil 2005; Schluter 2009; Nosil 2012). Speciation is facilitated when characters subject to divergent selection also affect reproductive compatibility—so-called magic traits (Gavrilets 2004). In species that use colorful signals in mating interactions, divergent selection on coloration may result in reproductive isolation (Seehausen et al. 2008). Here we test the hypothesis that divergent selection on coloration across an ecotone between mesic and xeric habitats decreases gene flow and promotes speciation in the Guadeloupean anole, *Anolis marmoratus*.

Ecological speciation is likely an important mechanism of diversification in the adaptive radiation of Caribbean *Anolis* lizards (Ogden and Thorpe 2002; Losos and Thorpe 2004; Thorpe et al. 2010; reviewed in Losos 2009). In this adaptive radiation ecological types, termed 'ecomorphs', are divergent primarily in body size and limb dimensions (Williams 1972; Beutell and Losos 1999). However, intraspecific variation, which may represent the initial phase of speciation, occurs primarily in adult male colour

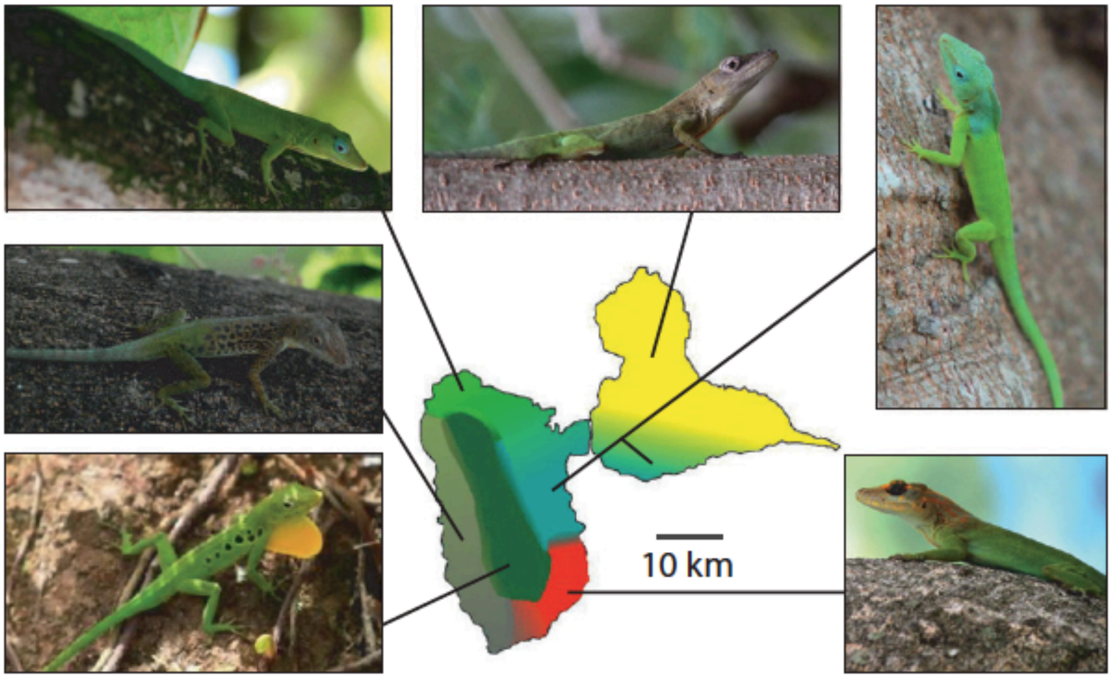

Figure 5_1. Adult males of the six named forms on the two main islands of the Guadeloupean Archipelago: Basse Terre (left) and Grande Terre (right). The forms are, clockwise from bottom left, *Anolis marmoratus alliaceus* (green with black spots); *A. m. girafus* (grey mottled); *A. m. setosus* (green with blue eyering and conical scales); *A. m. inornatus* (brown head and dull green-brown body, sometimes blue tail); *A. m. speciosus* (green with blue head and tail); and, *A. m. marmoratus* (red marbled head and blue tail). Areas of intergradation between forms are depicted as graduated shades of color. Photographs by Pierre Legreneur and André Schneider.

and pattern (Underwood and Williams 1959; Lazell 1972; Williams and Rand 1977; Thorpe et al. 2004) rather than in characters associated with ecomorph divergence (Knox et al. 2001). Geographically divergent body colour may result from drift in small populations, but is more often adaptive, resulting from ecological selection for background matching and crypsis (Rosenblum 2006), intra- or intersexual selection

(Stuart-Fox and Ord 2004; Stuart-Fox and Moussalli 2008), or both. Divergence in coloration may lead to speciation because the traits under selection potentially have a direct link to reproductive isolation in taxa, such as anoles, that use colorful signals in species recognition (Losos 1985, 2009; Ng and Glor 2011).

Geographic variation in male head and body coloration is extreme in *A. marmoratus* from the islands of the Guadeloupean archipelago in the Lesser Antilles. *Anolis marmoratus* comprises a polytypic species complex, with 12 subspecies described based primarily on the color and pattern of adult males (Lazell 1972). There are six parapatrically distributed subspecies on the two main islands, five on Basse Terre and two on Grande Terre, one of which is shared between islands (Fig. 5_1). In addition there are six allopatric populations on offshore islands that have variously been treated as subspecies or species (Lazell 1972; Schwartz and Henderson 1991; Schneider et al. 2001). Mitochondrial DNA variation among subspecies may be substantial, suggesting that populations may represent several stages along the speciation spectrum (Schneider et al. 2001). On the main island of Grande Terre, two subspecies are present, *A. m. speciosus* and *A. m. inornatus*. While these two subspecies are very similar both in mtDNA (Schneider et al. 2001) and in morphological dimensions (Knox et al. 2001), they differ dramatically in adult male color and pattern (Fig. 5_1). *Anolis m. speciosus* has a green body, a blue wash on the head with distinctive blue eyering and, often, a blue wash on the tail (Lazell 1972). In contrast, *A. m. inornatus* has a dull, yellow-green body, a browngreen head, a yellow or cream-coloured eyering and, often, a blue wash on the tail. Both subspecies have a similarly coloured yellow-orange dewlap, which is an extendable throat fan used in *Anolis* communication (Williams and Rand 1977; Jenssen 1978; Losos

2009). The females and juveniles are drably colored and show less distinct geographic variation (Lazell 1972).

The southwest of Grande Terre (Grands Fonds) is a hilly region composed of limestone laid onto a volcanic basement (Feuillet et al. 2002). The Grands Fonds is covered with broadleaf vegetation and transitions into the open, dry lowlands in the north and east of the island. The transition from mesic to xeric habitat is sharp and occurs on a scale of two to four kilometers. Clinal variation in lizard coloration is associated with habitat and occurs on a similar scale. The *speciosus* phenotype is found in the mesic Grands Fonds, whereas *inornatus* occupies the dry, open lowlands.

The pattern of green lizards in moist, forested habitat and dull-coloured (ranging from dull green to grey or brown) animals in open, dry habitat mirrors the general pattern found among species of *Anolis* and is thought to reflect adaptation for crypsis (Thorpe and Malhotra 1996; reviewed in Macedonia 2001; Losos 2009). However, variation in male coloration may also play a role in sexual selection. Blue coloration is typically conspicuous and involved extensively in lizard intraspecific communication (Cooper and Burns 1987; Zamudio and Sinervo 2000; Macedonia 2001; Robertson and Rosenblum 2009, 2010). Thus, the blue coloration of the head in *A. m. speciosus* is more likely associated with intra- or intersexual selection than with crypsis. Williams and Rand (1977) proposed that on single-species islands in the Lesser Antilles, such as Grande Terre, body and head color are the most salient visual cue for social communication or population recognition. Although the role of conspicuous colors in *Anolis* lizards remains unresolved, it is reasonable to hypothesize that both ecological and sexual selection may shape patterns of geographic color variation and represent important components of the speciation process in anoles.

Here we address three principal questions. First, is variation in adult male head and body color on Grande Terre consistent with hypotheses of ecological and sexual selection? Ecological selection for crypsis suggests that the reflectance of the body and head should fall within the reflectance of background vegetation and leaf litter, while sexual selection is expected to favor conspicuous color elements that stand out from the background. Second, is there a history of isolation between differentiated populations of *A. marmoratus* on Grande Terre? This question addresses the process of divergence—clinal variation in phenotype may result either from neutral processes of secondary contact and introgression among previously isolated populations, or from divergent selection in primary contact (i.e., parapatric divergence). Third, is the transition across the ecotone in phenotype (head and body color) associated with decreased gene flow? If divergence in coloration has a direct impact on reproductive isolation then we expect less gene flow per unit geographic distance across the ecotone than within habitats.

To answer these questions, we first analyzed phenotypic variation in coloration in relation to the background vegetation in each habitat. Second, we estimated historical demographic parameters for the two subspecies with a multilocus coalescent approach (Wakeley and Hey 1997; Hey and Nielsen 2004) using 10 sequenced anonymous nuclear loci to reconstruct the historical demographic parameters of divergence. Third, we examined population structure and gene flow using microsatellite markers assayed among populations across two transects spanning the ecotone and encompassing the full gamut of phenotypic variation on Grande Terre. Finally, we discuss the impact of our findings for understanding the processes of color evolution, local adaptation and ecological speciation.

Methods

Sampling and sequencing

We collected tissue samples (tail tips) from adult male lizards from 11 localities (Fig. 5_2, Table 5_1) across Grande Terre (n = 5–11, mean = 9) and preserved them in 95% ethanol. We sampled populations spanning the habitat and phenotypic transitions on Grande Terre along two transects: a south–north transect (Cocoyer to Port Louis) and a west–east transect from Cocoyer to Desvarieux. The S-N transect encompassed a sharp ecological transition between the mesic habitat in Lebaire and open habitat in Morne-a-L'Eau, which are only 2.4 km apart but are separated by an abrupt escarpment, whereas the W-E transect (Cocoyer to Desvarieux) traversed a shallower ecological transition from mesic to xeric habitats between Fouché and Chateaubrun (Fig. 5_2).

We extracted genomic DNA from tissue following standard protocols using QiagenTM DNAEasy kits. We created primers using Primer 3.0 (Rozen and Skaletesky 2000) for 40 haphazardly selected nuclear loci derived from 3072 Sanger sequence reads from a whole-genome library developed by the Broad Institute of MIT and Harvard. Primers were designed to yield sequences 300–500 base pairs (bp) in length. We screened all 40 primer sets on a test panel of six individuals (two each from Cocoyer, Port Louis and Desvarieux) and retained those that resulted in efficient single-copy gene amplification, as determined by single PCR products that showed only two alleles per variable site. Of 28 single copy loci, we retained the 10 loci that most reliably yielded

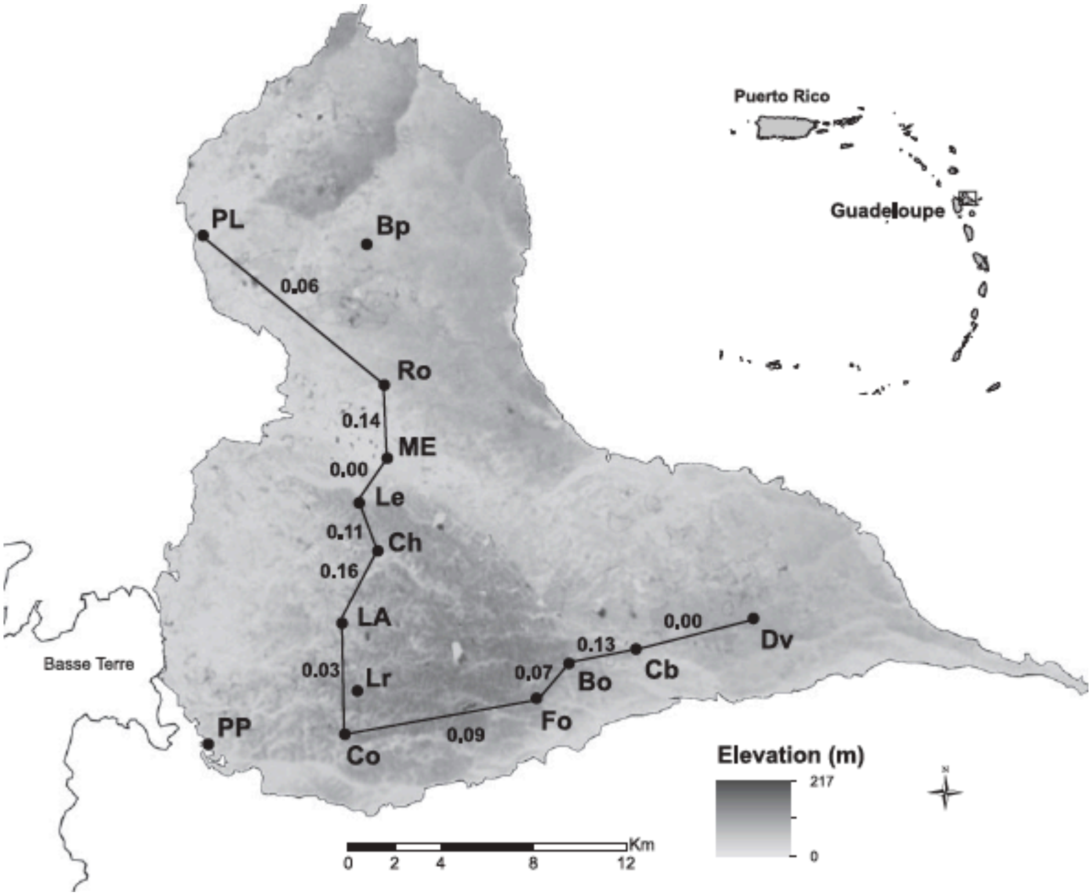

Figure 5_2. Sampling sites on Grande Terre, Guadeloupe. Locality abbreviations are given in Table 5_1. Genetic sampling sites are connected by dashed lines and values are G'_{ST} between adjacent populations estimated from microsatellite loci. Pointe-à-Pitre, Leroux, and Beauplan are sites where additional measures of animal reflectance were taken; reflectance measurements were not taken for animals from Port Louis or Les Abymes.

high-quality sequences. We sequenced both strands of each locus using BigDyeTM (Applied Biosystems) terminator chemistry on an ABI-3100 sequencer and, for subsequent analysis, deleted regions containing indels due to uncertain alignment and phasing. We used PHASE 2.1.1 (Stephens et al. 2001) to reconstruct haplotypes from sequence genotype data and, where phase at a particular polymorphic site was uncertain, those individuals were excluded from the analysis. We used IMgc (Woerner et al. 2007) to identify the largest, non-recombining region of sequence common to the greatest number of individuals.

Phenotypic analysis

We measured coloration of adult male lizards from 12 sites along two transects that spanned the ecotone on Grande Terre (Fig. 5_2). We characterized phenotypic variation in color by measuring reflectance of the dorsal head, eyering, dewlap, dorsal body and lateral tail using an Ocean Optics USB 2000 field-portable spectrometer. Reflectance values were recorded as per cent reflectance relative to a barium sulphate white standard using an Ocean Optics R400 UV-VIS reflectance probe attached to a DT-1000 tungsten halogen/deuterium UV-VIS light source (Ocean Optics, Inc., Dunedin, FL, USA). We also sampled reflectance of vegetation and leaf litter to characterize the visual background.

To visualize variation in color components along each transect, we used the hue angle estimate from the four-segment classification scheme of Endler (1990). To calculate hue angle, we used a custom python script (spec.py; http://github.com/ngcrawford/Coloration) to interpolate spectral reflectance values to 1 nm, smooth the spectral reflectance curve using a 100 nm hanning window, and calculate brightness, hue and chroma. We used TetraColorSpace (Stoddard and Prum 2008) to visualize lizard and background vegetation color as an avian predator with a U-type

Table 5_1. Summary of sampling localities on Grande Terre, including coordinates and elevation. Sample sizes denote number of individuals used in microsatellite analyses. DNA sample sizes denote number of individuals used in microsatellite analyses. Phenotypic sample size refers to number of male *Anolis marmoratus* sampled for each body part (head background, eyering, dewlap, lateral body and lateral tail). Phenotypic sample size with one value had the same value for all parts. Altitude is given in meters.

Locality	Code	Coordinates	Altitude	N (DNA)	N (color)
Port Louis	PL	16.42, -61.53	4	5	0
Rougeole	Ro	16.37, -61.46	17	9	5
Morne-a- l'Eau	ME	16.34, -61.46	2	9	4
Lebaire	Le	16.32, -61.47	61	8	11
Chazeau	Ch	16.30, -61.46	31	10	5
Les Abymes	LA	16.27, -61.48	48	9	0
Cocoyer	Co	16.23, -61.48	49	11	5, 0, 5, 5, 5
Fouché	Fo	16.24, -61.40	65	10	9
Bombo	Bo	16.26, -61.38	62	10	8, 9, 9, 9, 9
Chateaubrun	Cb	16.26, -61.36	26	10	5
Desvarieux	Dv	16.27, -61.31	49	5	10
Pointe-à-Pitre	PP	16.23, -61.53	6	0	28, 8, 27, 28, 28
Leroux	Lr	16.25, -61.47	49	0	35, 30, 35, 35, 35
Beauplan	Bp	16.42, -61.46	49	0	6

sensitivity might perceive the signal (Endler and Mielke 2005; Stoddard and Prum 2008). While most birds have a V-type retina, both U- and V-type retinas are sensitive to the UV so we chose to use the U-type model in our analysis to include the full range of reflectance from 300 to 700 nm. Also we used information on the visual sensitivity of *A. cristatellus* (Loew et al. 2002) to visualize color components as the lizards might perceive them.

Historical demography and subspecies population structure

Because the geographical context, time of divergence, and migration rate are central to understanding the process of divergence, we used a multilocus coalescent analysis to infer the demographic history of the two subspecies. We used the site variation data from 10 nuclear loci in an Isolation-with-Migration (IM) model as implemented in the program IMa2 (Hey and Nielsen 2007) to simultaneously estimate the time since divergence between subspecies (*t*), migration rates (*m1* and *m2*) between subspecies, and the effective population size for the ancestral (*qA*), and modern (*q1* and *q2*) populations. We pooled sequences from populations where eyering, head and body coloration were all consistent with subspecies description and excluded intergrade populations.

We ran a large number of preliminary IMa2 analyses with various priors, heating schemes, and number of chains to determine the parameters for our final run. Based on the observation of trend plots and ESS values, we found that MCMC runs with 40 chains and geometric heating with parameters set at -ha 0.96 and -hb 0.90 provided mixing as thorough as any other combination of heating parameters and number of chains that we explored (from 40 to 120 chains with geometric heating parameters of -ha from 0.96 to 0.99 and –hb from 0.75 to 0.90). We performed six independent replicate MCMC simulation runs for more than 28 million generations, each with 40 chains, geometric heating (-ha 0.96 and –hb 0.90), a different random number starting seed, a burn-in of 200,000 generations and priors of $q = 15$, $m = 100$ and t = 6. An infinite sites model was used and inheritance scalar priors were broad (10^{-3} to 10^{-10}). To convert parameter scalars to demographic units, we used a mutation rate per year of 0.5 substitutions per site per lineage per million years based on an estimate of synonymous substitution rate for the *Anolis carolinensis* genome (Janes et al. 2011). Generation time was assumed to be 1 year (Andrews 1976).

Microsatellite estimates of gene flow and population structure

To estimate fine-scale population genetic structure and gene flow among all pairs of populations, we screened seven microsatellite loci designed specifically for A. marmoratus. All loci were scored independently by two observers and cross-checked for accuracy (see Supp. Methods 5_1). We checked for violations of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP on the Web (v. 4.0.10; Raymond and Rousset 1995; Rousset 2008). We used GENALEX (Peakall and Smouse 2006) and custom code publicly available at http://github.com/ngcrawford/pypgen to calculate the following summary statistics: P, the proportion of polymorphic loci; H_S, Nei's unbiased estimate of heterozygosity (Nei 1987); and $a_{private}$, the number of alleles unique to a single population. To estimate the relative genetic differentiation among populations, and determine whether patterns of allele frequencies were associated with ecotones, geographic distance or both, we calculated Jost's D (Jost 2008) and G'_{ST} (Hedrick 2005) for all pairwise comparisons among populations using the web-based software SMOGD (Crawford 2010). We used an exact G-test for pairwise population differentiation (Goudet et al. 1996), as implemented in GENEPOP.

We also used the Bayesian clustering method in STRUCTURE 2.1 (Pritchard et al. 2000) to identify the number of populations that best explains patterns of allelic variation. Because of the shared recent ancestry and potential for mixed ancestry across populations, we employed the admixture model and the correlated alleles model with 100,000 generations as burn-in and run lengths of over two million generations for all values of K between 1 and 11. We used the log likelihood (-lnL) to determine the best estimate of *K*.

Results

Phenotypic variation across Grande Terre

Patterns in dorsal and head color display clinal variation across Grande Terre and are broadly concordant with ecological gradients (Fig. 5_3; Supp. Fig. 5_1). Between Lebaire (Le) and Morne-a-L'eau (ME) eyering color shifts from blue to yellow, body colour shifts from bright green to dull yellow-green and head color shifts from blue to brown. These populations are separated by only 2.4 km, and the phenotypic shift is coincident with the sharp ecological transition between the mesic closed habitat in the southwest (Le) and the scrubby open habitat found in the north (ME). Similarly, along the west to east transect, the ecological transition from mesic forest to open scrub occurs between Bombo (Bo) and Chateaubrun (Cb), over a distance of 2.9 km. Patterns in eyering, body, and head color follow a similar transition from the majority of individuals being blue, green and blue or green respectively, in the west, to yellow, dull yellow-green and brown in the east. Plots of color components in color space reveal that *A. m. speciosus* males in the southwest of Grande Terre are generally conspicuous, with even the green body coloration standing out from background (Fig. 5_4 and Supp. Fig. 5_1).

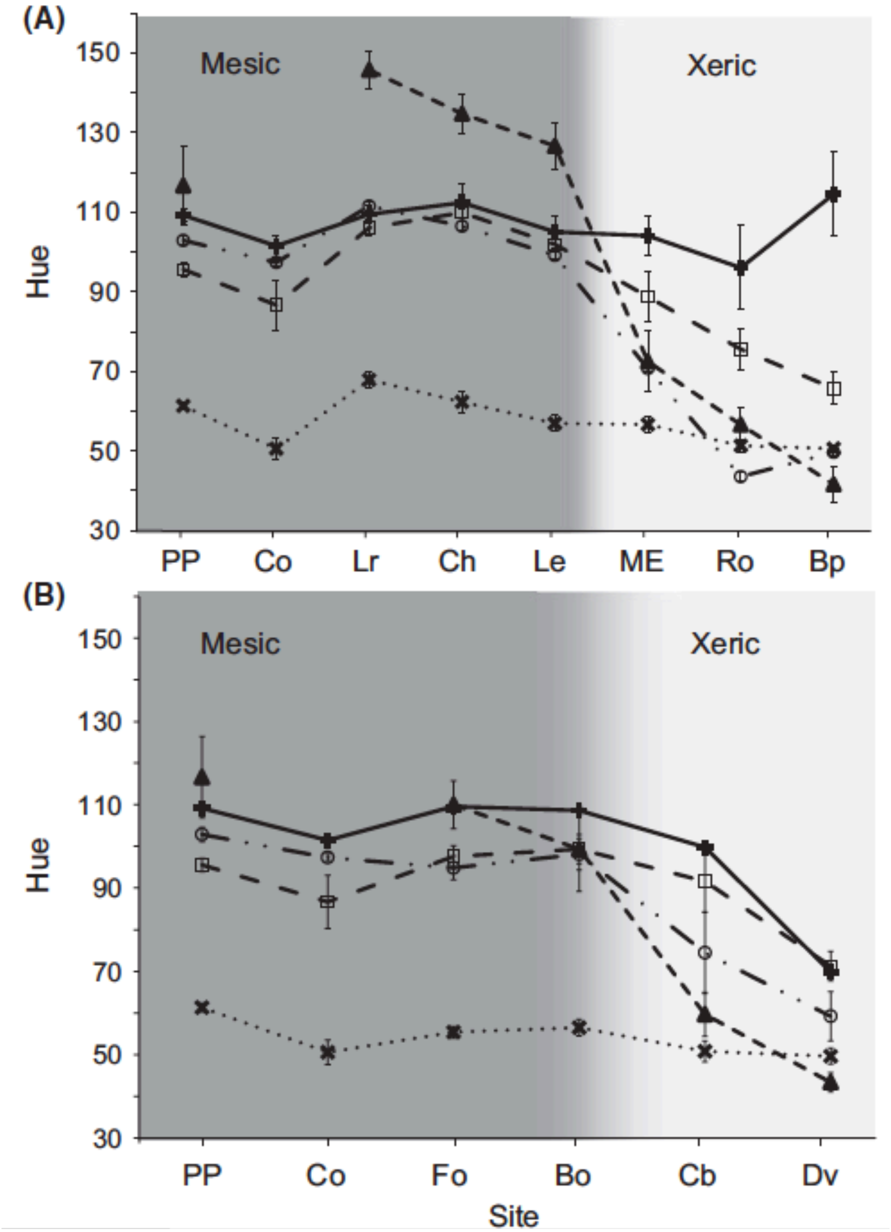

Figure 5_3. Average hue angle ± 1 SE for head background, eyering, dewlap, lateral body, and tail reflectance measured along (A) south to north and (B) west to east transects across an ecological gradient on Grande Terre, Guadeloupe. ▲ = eye ring; + = lateral tail; ○ = dorsolateral head (temporal region); ☐ = dorsolateral body (shoulder region); and X = center of the dewlap. The shaded background of the figures represents the approximate transition from mesic to xeric habitat. Locality defined in Table 5_1.

In contrast, *A. m. inornatus* male color components fall within, or much closer to, background reflectance (except for the dewlap and tail) making them generally cryptic. Dorsal coloration of female lizards overlaps with background reflectance consistent with female crypsis.

Coalescent-based inference of divergence parameters from sequenced nuclear loci

Sequences from the 10 nuclear loci ranged in size from 108 to 323 bp, and the complete data set comprises 2128 bp containing 56 variable sites (Table 5_2). Sequences mapped to non-repetitive, non-coding portions of the *A. carolinensis* genome (AnoCar 2.0), supporting the assumption that sequence polymorphism is selectively neutral.
The six independent MCMC runs of IMa2 converged on similar estimates of the model parameters, and ESS values for all parameters in each run were above 50, which, together with the trend plots, demonstrated sufficient mixing of the chains in each of the MCMC runs. We used the Lmode option in IMa2 to read in the genealogies from the output files of all six MCMC runs and summarize the results from a total of 1,731,863 genealogies (Table 5_3; Supp. Fig. 5_2). We visualized the results using the output
inornatus (Fig. 5_5; Table 5_3). More detailed information regarding IM run diagnostics are given in the Supp. Mat. 5_1.

Microsatellite genetic diversity and population structure

We analyzed seven microsatellite loci in 11 populations of *A. marmoratus* from Grande Terre. In seven populations, all microsatellite loci examined were polymorphic (*P* = 1.0), while the proportion of polymorphic loci remained high in the remaining four

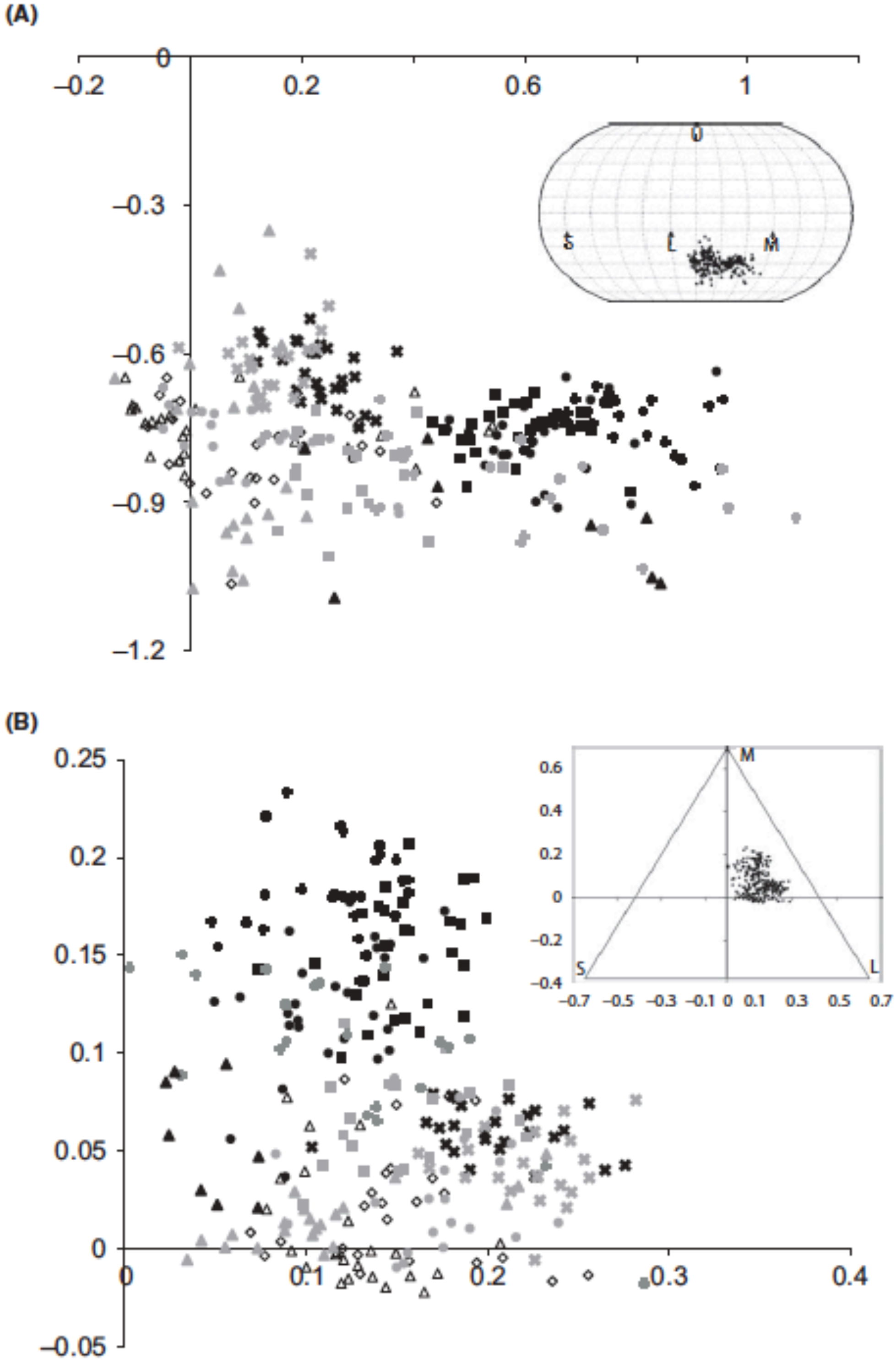

Figure 5_4. (A) Detail of Robinson projection (Endler et al. 2005) of lizard and visual background color components showing hue longitude on the x-axis and hue latitude on

Figure 5_4 continued: the y-axis. Inset shows position of all points in Robinson projection of complete tetrahedral color space. Δ = reflectance of visual background (vegetation and leaf-litter). ◊ = dorsolateral reflectance of females. Other symbols are reflectance from adult males. Grey symbols represent *A. m. inornatus*, black symbols represent *A. m. speciosus*: ▲= eye ring; + = lateral tail; ● = dorsolateral head (temporal region); ■ = dorsolateral body (shoulder region); X = center of the dewlap. (B) Plots of lizard color components and visual background projected on the S, M, L face of the color space tetrahedron (looking down along the UV axis). The scale and orientation of the S, M, L color space on the x-y plot is shown in the inset. Symbols are the same as above.

populations ($P = 0.86$; Table 5_4). The average number of genotypes analyzed per population was 8.7 and ranged from 5 to 11. Similarly, estimates of unbiased heterozygosity (HS) were consistently high across populations, and ranged from 0.566 (Port Louis) to 0.731 (Fouché). The average number of alleles unique to each population ($a_{private}$) was 1.7, and ranged from 0 (Les Abymes) to 5 (Fouché). In most populations, however, aprivate was consistently low (≤ 2), except in Bombo and Fouché ($a_{private} = 3$ and 5, respectively).

Microsatellite variation shows low levels of genetic differentiation among populations. Multilocus estimates of G'_{ST} ranged from 0 to 0.182 and D ranged from 0 to 0.171 (Table 5_5). G'_{ST} values between populations in the west–east transect (Cocoyer to Desvarieux) were similar to estimates between populations in the south–north transect (Cocoyer to Port Louis). Only two multilocus estimates of population differentiation

Table 5_2. Characteristics of sequenced nuclear loci. Mutation rate (μ) per year is the per-locus mutation rate used to convert IM parameter scalars to demographic parameters. Mutation rate per site per million years per lineage of 5 × 10^{-7} is taken from the analysis of Janes et al. (2011). The geometric mean of the per-locus mutation rates is 1.01 × 10^{-4}. The position in the *A. carolinensis* genome reflects high-scoring BLAST alignments of locus sequence reads to the AnoCar2.0 genome build. No loci mapped to exon regions.

Locus	Size (bp)	Variable Sites	$N_{speciosus}$, $N_{inornatus}$	μ per year	Position in *Anolis carolinensis* genome
1FA3	137	5	28, 18	0.000069	chr3:109,228,783-109,228,909
1FA4	286	9	28, 16	0.000148	Chr2:73, 603,309-73,603,389
1FA5	153	2	26, 18	0.000077	chrUn_GL343268:21,075-21,208
1FA7	108	7	28, 16	0.000054	chr1:45,032,731-45,032,808
1FB1	169	7	24, 8	0.000085	chr4:99,942,237-99,942,407
1FB3	202	8	24, 12	0.000101	chr6:24,722,277-24,722,488
1FC8	323	10	24, 10	0.000162	chrUn_GL343584:350,635-350,954
10FB3	201	4	28, 16	0.000101	chrUn_GL343289:1,546,328-1,546,516
10FB8	320	2	30, 14	0.000160	chr5:103,583,017-103,583,215
10FB12	229	2	26, 10	0.000115	chr1:16,986,237-16,986,465

were significant after Bonferroni correction. These were Bombo-Chateaubrun (G'_{ST} = 0.145; Exact G-test $p = 0.0009$) and Bombo-Lebaire (G'_{ST} = 0.118; Exact G test $p = 0.0007$). Exact G-tests for allele frequency differences among pairs of populations for each locus revealed that a single locus (12RF3) appeared to be driving the pattern; after Bonferroni correction for multiple comparisons, only comparisons for that locus and for the two population comparisons above were significant ($p < 0.02$).

Table 5_3. Summary of the six independent IMa2 runs showing estimates of demographic parameters for the divergence histories for *Anolis m. speciosus* and *A. m. inornatus*. The values for the peak (HiPt), as well as the Low (95%Lo) and High (95%Hi) points of the 95% confidence interval are given.

Measure	N_e (ancestral)	N_e (*inornatus*)	N_e (*speciosus*)
HiPt	369,389	1,136,534	135,598
95% Lo	126,247	565,773	32,731
95% Hi	1,079,800	9,002,494	8,965,087

Table 5_3 continued.

Measure	2*Nm* (*inornatus* → *speciosus*)	2*Nm* (*speciosus* → *inornatus*)	Time since divergence (years)
HiPt	2.64	21.36	650,872
95% Lo	0.37	7.87	351,621
95% Hi	11.62	423.2	14,476,309

Table 5_4. Summary statistics of genetic diversity for seven microsatellite loci used in this study. *N*, total number of individual genotypes; *P*, proportion of polymorphic loci; $a_{private}$, number of alleles unique to a single population; H_S, Nei's unbiased heteozygosity.

Population	Scored Loci	*N*	*P*	H_S (±1SEM)	a_{private}
Bombo	7	10	1.00	0.624 (0.236)	3
Chateaubrun	7	10	1.00	0.651 (0.257)	2
Chazeau	7	10	1.00	0.675 (0.246)	2
Cocoyer	7	11	1.00	0.654 (0.222)	1
Desvarieux	7	5	0.86	0.599 (0.273)	1
Fouché	7	10	1.00	0.731 (0.156)	5
Lebaire	7	8	0.86	0.618 (0.278)	1
Les Abymes	7	9	1.00	0.639 (0.204)	0
Morne-a-L'eau	7	9	0.86	0.650 (0.284)	2
Port-Louis	7	5	0.86	0.566 (0.288)	1
Rougeole	7	9	1.00	0.628 (0.245)	1

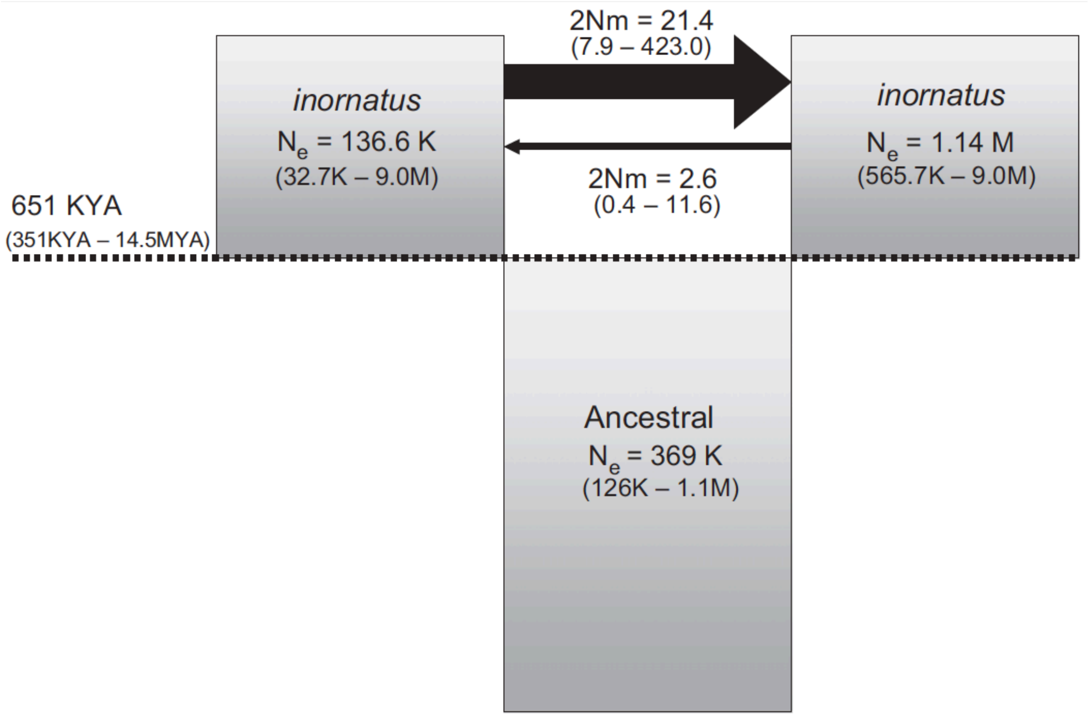

Figure 5_5. Divergence histories for the two subspecies on Grande Terre. Effective population size (N_e with bounds of 95% highest posterior density) for *Anolis m. speciosus*, *A. m. inornatus* and the ancestral population are shown in the shaded boxes (boxes not drawn to scale). Time is on the vertical axis, with recent time at the top, and the estimated divergence time (with 95% HPD) is shown. Gene flow is indicated by arrows indicating the direction of gene movement, and values for 2*Nm* (with 95% HPD) are shown.

Overall, there was no pattern of isolation by distance (IBD) (Fig. 5_6). The sharp phenotypic transition between *A. m. speciosus* and *A. m. inornatus* that occurs between Lebaire and Morne-a-L'eau is not accompanied by increased G'_{ST} values. However, the phenotypic transition between Bombo and Chateaubrun is associated with increased G'_{ST}

Table 5_5. Pairwise comparisons of G'_{ST} (bottom left) and Jost's D (top right) estimated from seven microsatellite loci among all populations. Populations are ordered North to South, West to East. Multilocus G'_{ST} *p*-values for genotypic differentiation resulting from an exact G-test (Goudet et al. 1996) as implemented in GENEPOP are denoted as follows: $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. Population abbreviations are as in Table 5_1.

Pop.	PL	Ro	ME	Le	Ch	LA	Co	Fo	Bo	Cb	Dv
PL	-	0.049	0.091	0.000	0.007	0.161	0.017	0.000	0.035	0.105	0.006
Ro	0.063	-	0.029	0.030	0.163	0.099	0.010	0.101	0.025	0.108	0.000
ME	0.140	0.182*	-	0.038	0.032	0.171	0.024	0.021	0.013	0.025	0.025
Le	0.000	0.062	0.111*	-	0.061	0.077	0.003	0.049	0.035	0.024	0.001
Ch	0.112	0.169*	0.085	0.120	-	0.124	0.010	0.018	0.128	0.098	0.002
LA	0.164	0.124	0.163	0.101	0.149*	-	0.038	0.065	0.056	0.066	0.038
Co	0.025	0.042	0.050	0.018	0.070	0.064	-	0.044	0.050	0.013	0.001
Fo	0.088	0.142	0.056	0.095	0.067	0.123	0.064	-	0.092	0.017	0.001
Bo	0.072	0.113	0.071	0.118***	0.167**	0.123*	0.089*	0.149*	-	0.099	0.004
Cb	0.128	0.138*	0.065*	0.077	0.098	0.098*	0.035	0.063	0.145***	-	0.000
Dv	0.000	0.051	0.053	0.000	0.043	0.056	0.005	0.038	0.030	0.000	-

although, as noted above, the pattern may be caused by a single locus. Overall, G'_{ST} values across the ecotone are not higher than values within habitats when standardized for geographic distance (Fig. 5_6). Pairwise estimates of gene flow (*Nm*) derived from G'_{ST} are all substantially greater than one suggesting that gene flow between adjacent populations is high enough to homogenize neutral genetic variation.

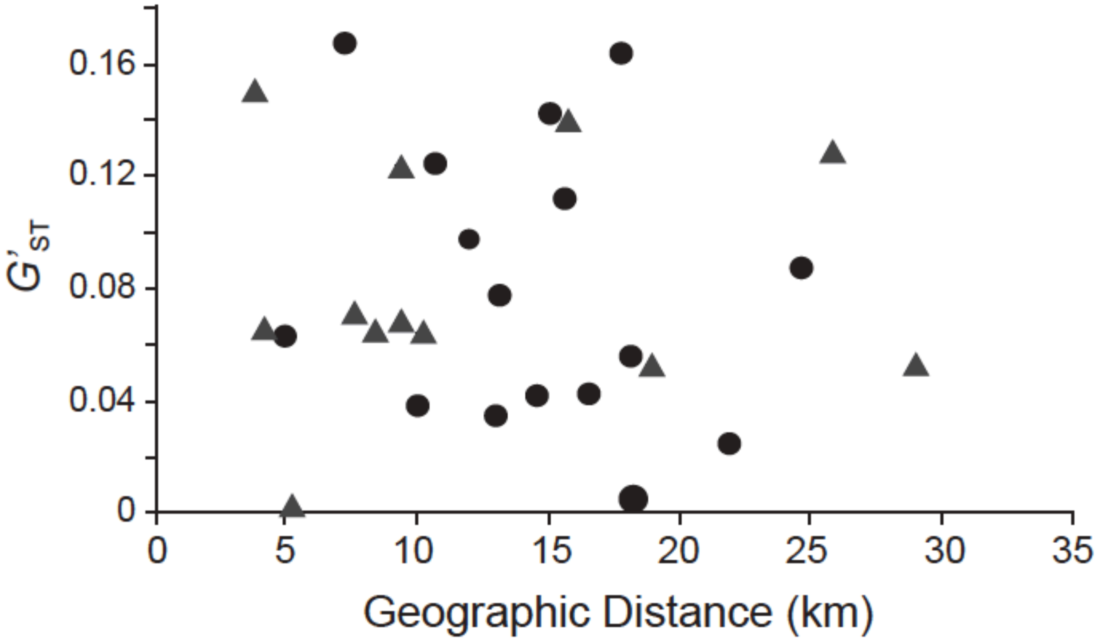

Figure 5_6. G'_{ST} plotted against geographic distance within (triangles) and between (circles) habitats. No pattern of isolation by distance is evident (Mantel test $p = 0.5$) nor is G'_{ST} among populations between habitats higher than G'_{ST} within habitats over similar geographic distances.

The pattern of low genetic differentiation among populations was further supported by the STRUCTURE analysis (Fig. 5_7). A model with two populations ($K = 2$) had the highest likelihood (lnL = - 2132) of the 11 models examined ($K = 1–11$). The likelihood of a model with two populations was significantly better than the likelihood of a model with one population (lnL = - 2145; $D = 25.8$; chi-square test, d.f. = 1; $p < 0.001$), and likelihoods for all other values of K were substantially worse (lnL - 2209 to - 2620). However, an examination of the probability of assignment to either of the two clusters for $K = 2$ shows no distinguishable pattern (Fig. 5_7). Most individuals were classified as belonging to a single population with high probability. Three individuals from Chazeau, one from Les Abymes, and one from Port Louis were assigned to a second genetic cluster with fairly high probability. However, these five individuals span the breadth of the S-N transect and do not form a coherent cluster in space. With the exception of these five individuals, all others are assigned with high probability to a single nearly homogeneous population.

Discussion

Local adaptation in multiple color elements of A. marmoratus

The results from our phenotypic analysis are concordant with the hypothesis that ecological and sexual selection mediate color evolution in *A. marmoratus*. As expected, patterns in dorsal and head color vary clinally across the island, and phenotypic transitions coincide with the mesic–xeric ecotone (Fig. 5_3). Color space plots reveal that

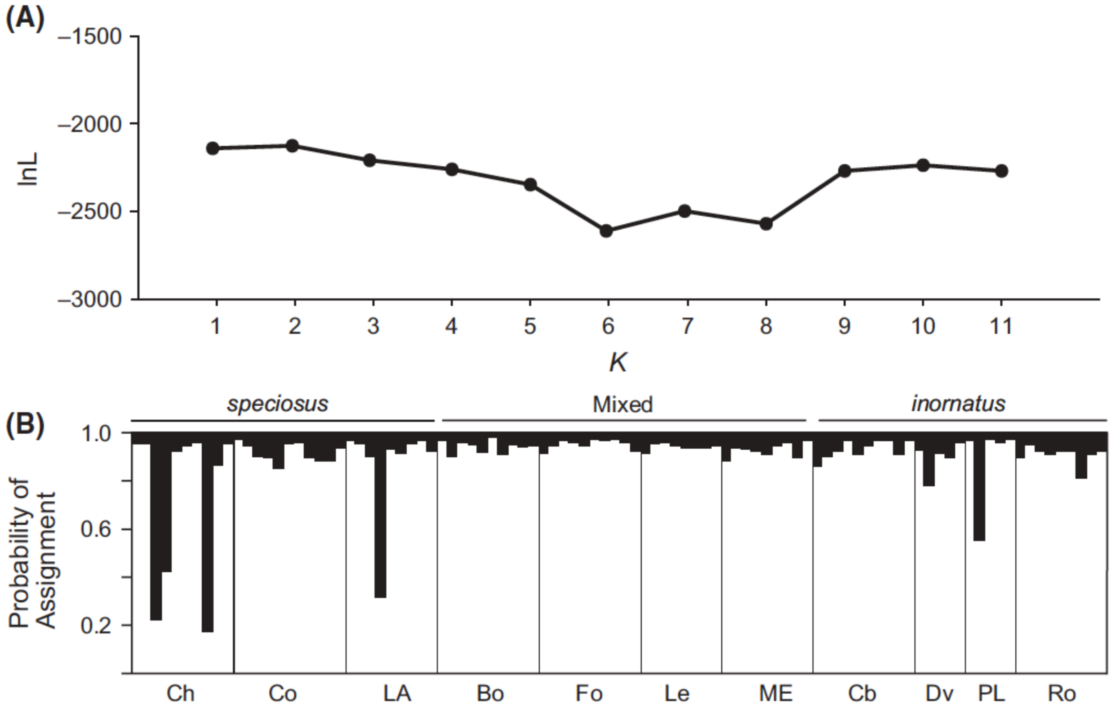

Figure 5_7. Structure analysis. (A) lnL for *K* = 1–11 populations. (B) Probability of assignment of individuals to either of two populations.

the head and body coloration of *A. m. inornatus* overlap with background reflectance (Fig. 5_4) consistent with crypsis due to background matching (e.g., Hoekstra et al. 2004; Endler et al. 2005; Rosenblum 2006). In contrast, plots of color components reveal that *A. m. speciosus* is conspicuous in its environment, as individuals show little overlap with background reflectance in color space (Fig. 5_4). Even the green body coloration stands out from the background in this subspecies. Sexual selection may favor general conspicuousness when predation risk from visual predators is low (Reznick and Endler 1982; Kemp et al. 2009), and we suspect that the predation by avian predators may be driving this pattern. Our results strongly suggest that divergent selection for crypsis (in open habitats) and conspicuity (in closed habitats) are mediating evolution in multiple colorful elements in *A. marmoratus.*

The history of divergence among subspecies

The observed colour variation among populations may result either from secondary contact and introgression or from primary divergence, without geographic isolation, due to divergent selection (Endler 1977). Our analysis of historical demography strongly supports the hypothesis that divergent natural selection across the ecological gradient generated clinal patterns of phenotypic divergence in this complex (classic parapatric divergence of Mayr 1947). Furthermore, the analysis of nuclear sequence variation in an Isolation-with-Migration (IM) model found a strong signal of migration between subspecies and large effective population sizes throughout their history, supporting a model of divergence with gene flow (Fig. 5_5).

Our results are consistent with in situ diversification of *A. marmoratus* on Grande Terre. It is not surprising that the alternative model of geographic isolation followed by secondary contact is not strongly supported. Grande Terre is a geologically young island, having formed through the deposition of limestone platforms during the Pleistocene, building east and northward from the Grands Fonds in the southwest of the island, and there is limited evidence for periods of geographic separation between regions of the island (Feuillet et al. 2002; Komorowski et al. 2005). The time since divergence, estimated at about 650 KYA, suggests that the populations on Grande Terre diverged well after the formation of the island. Finally, there is no sign of a population bottleneck or founder event, and the ancestral population size for these subspecies is inferred to be large, with N_e estimated at 369,000 individuals. This is not surprising given that anoles are good dispersers and that population densities, especially in the Lesser Antilles, can be extraordinarily high; more than 32,000 individuals per hectare have been estimated for *A. trinitatis* from St. Vincent (Hite et al. 2008). Thus, all indications are that phenotypic divergence among subspecies on Grande Terre occurred due to divergent selection in primary contact.

Phenotypic variation is maintained despite near panmixia at neutral loci

Our finding that *A. m. inornatus* is cryptic in its environment, whereas *A. m. speciosus* is conspicuous suggests a strong role for divergent selection and, consequently, the potential for genomic divergence among subspecies. Indeed, previous research in anoles and other lizards has demonstrated that neutral genetic structure can be stronger across ecotones than within the same habitats (Ogden and Thorpe 2002; Rosenblum 2006; Thorpe et al. 2010). However, we find little indication of reduced gene flow among phenotypically differentiated populations of *A. marmoratus* on Grande Terre suggesting that gene flow and recombination are high enough to homogenize genomic variation except at loci that have a strong effect on fitness.

Our finding of high gene flow among populations is somewhat surprising given that phenotypic variation among populations is mainly in the coloration of adult males, a character that may impact reproductive isolation in highly visual animals in which mating success is related to social signaling. A possible explanation for the lack of genetic structure is that divergence among populations may be so recent that genetic variation at marker loci is not at migration—drift equilibrium (Wright 1943). At equilibrium, we expect to see a pattern of IBD if dispersal is limited relative to the geographic scale of analysis (Slatkin 1993). Our genetic data are not consistent with a pattern of IBD (Fig. 5_6), but given the temporal depth of divergence of the *A. marmoratus* subspecies on Grande Terre (~650 KYA corresponding to an equivalent number of generations), we expect populations to be at migration—drift equilibrium. The lack of genetic structure, therefore, probably reflects rates of gene flow and recombination that are high enough to homogenize allele frequencies at neutral loci, while frequencies at loci affecting coloration have diverged.

Anole coloration displays little phenotypic plasticity (Steffen et al. 2010) and therefore phenotypic variation maintained along ecological gradients in the face of high gene flow probably reflects divergence at a small number of loci under selection (e.g., Mullen and Hoekstra 2008; Freedman et al. 2010; Rosenblum and Harmon 2011). These loci remain differentiated between phenotypically divergent populations despite high levels of gene flow across the genome. Although color variation loci are not driving the evolution of reproductive isolation (e.g., Turner et al. 2005), they may be viewed as 'adaptation islands', as they underlie the evolution of a strong phenotypic cline in the face of high gene flow at loci that are selectively neutral. This view of a porous genome (Feder 1998; Gavrilets and Vose 2005) suggests that strong selection can overcome gene flow to fix adaptive loci while gene flow and recombination homogenize variation across loci that are selectively neutral.

Implications for divergence with gene flow, ecological speciation and adaptive radiation

Because of the strong phenotype-habitat correlation observed across *Anolis*, we might expect an important role for ecological speciation in the adaptive radiation of anoles. Some species of *Anolis* show evidence for stages in the process, but we do not yet have clear examples of complete ecological speciation (i.e., largely or fully reproductively isolated species). For example, *A. roquet* from Martinique shows patterns consistent with ecological speciation in that levels of gene flow across an ecological transition are lower than among previously isolated populations that currently occupy the same habitat (Ogden and Thorpe 2002; Thorpe et al. 2010). However, reproductive isolation is not sufficient to allow differentiated species to coexist, a necessary step in speciation within islands. Similarly, while *A. m. inornatus* and *A. m. speciosus* clearly represent a case of divergence with gene flow, likely due to divergent selection on multiple traits, we see no sign of reproductive isolation. Thus, taxa from geographically complex islands in the Lesser Antilles appear to represent cases in which the processes of population divergence are revealed, but where speciation has stalled at different stages. What, then, inhibits ecological speciation on small islands?

In models of ecological speciation, reduction in gene flow between divergent phenotypes is an important step in transforming ecotypic variation into reproductively isolated species (Kirkpatrick and Ravigné 2002; Nosil 2008; Hendry 2009). The evolution of reproductive isolation in the face of gene flow is difficult because recombination randomizes associations between genes under divergent selection and those that affect reproductive isolation (Felsenstein 1981). However, this selection/recombination antagonism disappears when the loci under selection pleiotropically affect reproductive isolation (Rice and Hostert 1993; Gavrilets 2004; Mallet et al. 2009; Servedio et al. 2011).

Are there traits in *Anolis* that are under divergent selection and that may pleiotropically result in reproductive isolation—so-called magic traits (Gavrilets 2004)? There is strong evidence that dewlap coloration is important in species recognition in *Anolis* (Rand and Williams 1970; Losos 1985; Nicholson et al. 2007; Ord and Stamps 2009; reviewed in Losos 2009) and sympatric species nearly always differ in dewlap color. It has been suggested that tight dependence on dewlap coloration combined with species-specific display patterns may explain the near absence of hybridization among sympatric species (Losos 2009). If adaptive divergence in dewlap coloration occurred among populations (e.g., local adaptation for effective signaling in different light environments), reproductive isolation may follow as a consequence. There is some evidence that divergence among populations in dewlap coloration is related to decreased gene flow (Ng and Glor 2011), but there is little evidence that dewlap coloration is a direct target of divergent selection prior to speciation (Nicholson et al. 2007). Williams and Rand (1977) hypothesized that head and body coloration also may be important components of the mate recognition system but we find no evidence to support such a link. Finally, an analysis of multiple quantitative traits associated with reduced gene flow across an ecotone in *A. roquet* identified several characters that covary across the ecotone (Thorpe et al. 2012). However, it does not seem likely that those characters, which may be under divergent selection (e.g., number and size of scales), have a direct impact on reproductive isolation. Thus, there is no evidence to date for magic traits in anoles that may be both the target of divergent selection and also affect reproductive compatibility.

Sexual selection is likely an important cause of phenotypic evolution in anoles (reviewed in Losos 2009), and variation in female preference associated with habitat could provide a mechanism for speciation via sexual selection by female choice (Kirkpatrick and Ravigné 2002; Seehausen et al. 2008). However, the prevailing view is that there is no evidence for female preference in *Anolis*, or any other lizard for that matter (Stamps 1983; Andrews 1985; Tokarz 1995; Lailvaux and Irschick 2006), and the lack of assortative mating suggested by our data is consistent with that view. Rather, sexual selection in *Anolis* appears to take the form of male–male competition for territory, and divergent male coloration may evolve in the context of intrasexual interaction without concomitant evolution of assortative mating. In the absence of geographic isolation, therefore, divergent sexual selection may not have a large impact on the initial stages of adaptive radiation of anoles, although it may still play a role in reinforcement.

Our findings suggest that loci controlling head and body coloration are not involved in reproductive compatibility. For reproductive isolation to evolve without direct selection on loci that affect reproductive isolation, linkage disequilibrium between reproductive compatibility loci and loci under divergent selection must evolve (Felsenstein 1981; Rice and Hostert 1993; Kirkpatrick and Ravigné 2002; Gavrilets 2003, 2004). The evolution of linkage disequilibrium among disparate loci is unlikely given the high rates of gene flow and large effective sizes that we estimated for divergent populations on Grande Terre. Therefore, some degree of geographic isolation or IBD may be necessary for the evolution of linkage disequilibrium and speciation in *Anolis*.

Losos and Schluter (2000) identified an evolutionary species–area relationship for

Anolis whereby speciation occurs only on islands above a threshold size. This pattern may be general; Kisel and Barraclough (2010) surveyed a wide variety of taxa on oceanic islands and found a similar evolutionary species–area relationship. Both sets of authors suggested that gene flow inhibited speciation on islands below a threshold size. Our findings are consistent with that hypothesis, but we point out that, at least in *Anolis* and probably other taxa as well, there appears to be a gradation of differentiation with island size—small, topographically simple islands contain single, monotypic species while intermediate sized islands often show minor geographic variation within a single species, and similar-sized volcanic islands, with substantially more topographic complexity, harbor polytypic species, sometimes with extreme geographic variation (Losos and Schluter 2000). Notably, this pattern is reflected within the *A. marmoratus* species complex, with six subspecies on the volcanic island of Basse Terre and two on the similarly sized, but more topographically simple, island of Grande Terre. Such continuity of pattern suggests continuity of process and highlights the fact that the homogenizing effects of gene flow may be overcome by strong divergent selection to produce substantial phenotypic variation over small spatial scales. With sufficient isolation from gene flow, linkage disequilibrium between loci under divergent selection and those controlling assortative mating could evolve. Without geographic isolation, the balance between gene flow and selection will determine where along the speciation spectrum population divergence comes to rest.

Conclusions

Male *Anolis marmoratus* on the island of Grande Terre show striking phenotypic differentiation in multiple colorful elements. Our analysis of coloration in relation to habitat suggests that sexual selection for conspicuousness is primarily responsible for the striking blue and bright green coloration of males in the mesic southwest of the island, whereas in the open habitat of the north and east, ecological selection for crypsis has resulted in the drab coloration of males. Our multilocus coalescent and microsatellite analyses support a model of divergence with gene flow and high contemporary rates of gene exchange. Thus, while phenotypic divergence in adult male coloration is striking, we find no indication that reproductive divergence has occurred as a consequence. This suggests that the anoles on Grande Terre represent an early stage along the speciation continuum and that assortative mating is not complete. High gene flow among populations may inhibit further progress toward speciation, particularly if the loci affecting fitness in the two habitats are not the same as loci affecting traits involved in reproductive isolation; thus, these populations are likely at a stable endpoint that reflects a balance between divergent selection and gene flow. This migration–selection antagonism may explain the evolutionary species–area relationship noted by Losos and Schluter (2000) and also explain the fact that ecological speciation appears to have repeatedly stalled at different stages on topographically complex islands in the Lesser Antilles (Losos 2009; Thorpe et al. 2010).

Our findings are consistent with strong divergent natural and sexual selection acting on coloration among populations. Similar selection may be playing out in other portions of the *A. marmoratus* species complex but we do not yet know whether stronger selection is correlated with reduced gene flow as predicted by models of ecological speciation. The *A. marmoratus* species complex provides a rare opportunity to examine the interaction of gene flow, geographic isolation, and ecological and sexual selection on population divergence and speciation across a wide portion of the speciation spectrum. Analyses of population divergence across the steeper ecological gradients on the older volcanic island of Basse Terre, and analyses of populations isolated on small offshore islands, will allow additional tests of ecological speciation and identify the factors that determine the outcome of population divergence in response to ecological and sexual selection.

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Appendix

Chapter 1

Supp. Table 1_1. Summary information is given for each locality visited for this study. Mean annual temperature (MAT) was extracted from the WorldClim database (Hijmans et al. 2005). Mountain chains are the Cordillera Central (CC) and the Sierra de Baoruco (SB) (Supp. Fig. 1).

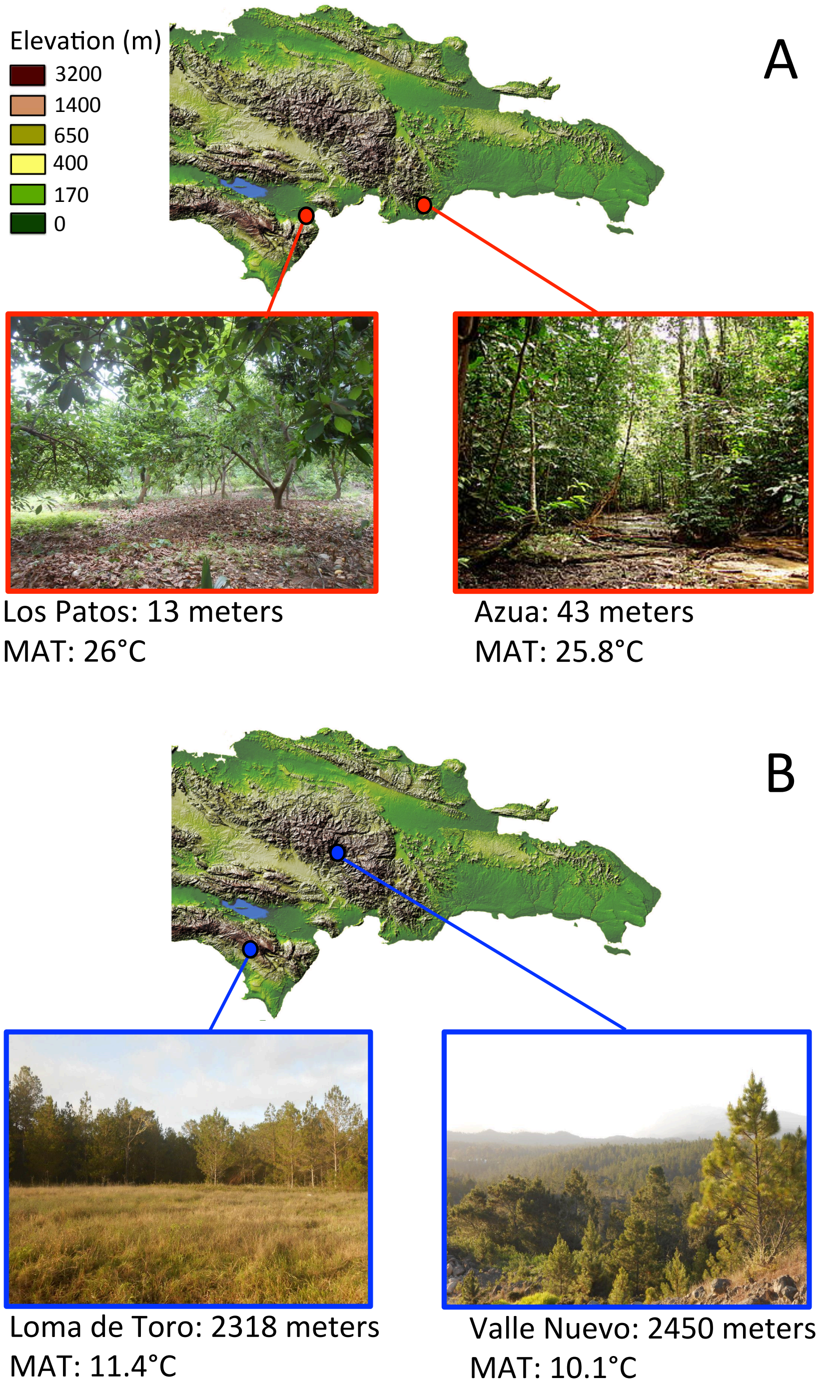
Locality	Mountain Chain	Coordinates	Elevation (m)	MAT (°C)
Caamaño	CC	18º 26.119' N, 70º 35.546' W	43	26.9
Valle Nuevo	CC	18º 43.800' N, 70º 36.000' W	2450	10.1
Los Patos	SB	17º 57.300' N, 71º 11.288' W	13	26.0
Loma de Toro	SB	18º 17.244' N, 71º 42.749' W	2318	11.4

Supp. Table 1_2. Summary statistics evaluating thermal habitat and thermoregulation at each locality. Thermal habitat quality, d_e, is given for rocks and trees and across both perch types. % below, within, and above refer to the percent of operative temperatures that fell below, within, or above the preferred temperature range, respectively. Mean body temperature (Tb) and the preferred body temperature are given in °C ± 1 s.e.m. Thermoregulation was assessed through the degree of behavioral thermoregulation, d_b, and the effectiveness of thermoregulation, *E*, between low and high elevation sites. An asterisk denotes the perch type with the highest thermal quality at each elevation. Values closer to zero indicate a higher degree of thermoreulation (d_b) and a greater thermoregulatory efficiency (*E*).

Locality	Elevation	d_e (rocks)	d_e (trees)	% below (rocks, trees)	% within (rocks, trees)	% above (rocks, trees)	T_b (°C)	T_p (°C)
Caamaño	43	2.37	2.09	33.2, 34.0	15.1, 13.7	51.7, 52.3	30.3 ± 0.4 (104)	30.2 ± 0.3 (17)
Valle Nuevo	2450	8.23	8.87	53.4, 91.4	11.0, 6.3	35.6, 2.3	29.7 ± 0.3 (58)	29.3 ± 0.9 (19)
Los Patos	13	4.79	2.14	23.2, 24.9	19.4, 28.1	57.4, 46.7	29.7 ± 0.3 (83)	29.4 ± 0.3 (21)
Loma de Toro	2258	9.84	10.11	84.6, 99.1	7.8, 0.7	7.5, 0.2	28.9 ± 0.3 (68)	30.7 ± 0.3 (23)

Supp. Table 1_3. Morphological differences between low and high elevation populations of *Anolis cybotes*. *p*-values are provided for elevation effects on each trait, based on an ANCOVA with elevation as the fixed effect and body size (snout-vent length, SVL) as the covariate. Comparisons for wild caught adults are given of the left and comparisons among laboratory-reared lizards from a common garden experiment are given on the right. Traits with significantly different intercepts among elevations are highlighted in bold. †: d.f. = 1, 96.

	Wild Caught		***Common Garden***	
Trait	$F_{1,\,97}$	p	$F_{1,\,18}$	p
Head Height	155.7	**<0.001**	33.7	**<0.001**
Head Width	46.4	**<0.001**	2.7	0.119
Femur	66.8	**<0.001**	17.8	**0.001**
Tibia	144.7	**<0.001**	19.3	**<0.001**
Metatarsus	41.9	**<0.001**	0.3	0.146
IV Toe	$2.4^{\dagger}$	0.125	0.9	0.366



Supp. Fig. 1_1. Map showing sampling localities. Low elevation localities (A) are in red, and high elevation localities (B) are in blue. Photographs of habitat, as well as mean annual temperature (MAT) are also given. Coordinates are given in Supp. Table 1_1.

Chapter 2

Supp. Table 2_1. Details of the literature survey. The publication year, lead author, journal, organisms studied, and geographic region examined are given. For each study, we recorded the number and types of niche dimensions examined. The niche dimensions considered were: climate, diet, habitat, and time. The outcome of the study (niche conservatism, NC; niche divergence, ND) is also given. The citation number (Cit. #) corresponds to the citation order in the Works Cited section.

Cit. #	Year	Lead Author	Organisms	Geographic Scale	# Axes	Types	Outcome
1	2014	Acevedo	hares	Southern Europe	1	climate	NC
2	2014	Amano	flowering plants	United Kingdom	2	climate, time	NC, ND
3	2014	Anacker	plants	California Floristic Province	3	climate, habitat, time	NC, ND
4	2014	Angulo	desert shrub	Southern North America	1	climate	ND
5	2014	Assis	brown alga	North America	1	climate	NC
6	2014	Broennimann	spotted knapweed	North America, Europe	1	climate	NC
7	2014	Escoriza	frogs	North Africa, Southern Europe	2	climate, habitat	NC, ND
8	2014	Feindt	damselfy	Mexico, Central America	1	habitat	NC
9	2014	Glennon	diploid and polyploid plants	North America, Europe	1	climate	NC
10	2014	Guarnizo	frogs	Andes	1	climate	NC
11	2014	Hipsley	worm lizard	North America	1	climate	ND
12	2014	Hopkins	invertebrates	global	1	habitat	NC, ND

Supp. Table 2_1 continued.

13	2014	Kolanowska	orchid	Asia, Central America, South America	1	climate	NC
14	2014	Kolanowska	orchid	Africa, South America	1	climate	NC
15	2014	Kostikova	buckwheat	Southwestern North America	1	climate	ND
16	2014	Kostikova	buckwheat	global	1	climate	ND
17	2014	Pearman	birds	Europe	3	climate, diet, habitat	ND
18	2014	Perez	myrtle	Chile	1	climate	NC
19	2014	Rodrigues Marques	bromeliads	Brazil	1	climate	NC
20	2014	Schmidt	microbes	global	1	habitat	NC
21	2014	Sillero	fruit fly	North America	1	climate	ND
22	2014	Strubbe	birds	Europe	1	climate	NC
23	2014	Tarroso	vipers	Northern Spain	2	climate, habitat	ND
24	2014	Tkach	green root parasite plants	Arctic	1	climate	NC, ND
25	2014	Zhu	western conifer seed bug	North America, Europe, Asia	1	climate	NC
26	2014	Zink	birds	North America	1	climate	NC
27	2013	Ahmadzadeh	lizard	Eastern Mediterranean, Anatolia	1	climate	NC, ND
28	2013	Alexander	scarole	Europe, Asia	2	climate, time	NC, ND
29	2013	Arbelaez-Cortes	hummingbird	Western Mexico	1	climate	NC
30	2013	Di Febbraro	gray squirrel	North America, Northern Europe	1	climate	ND

Supp. Table 2_1 continued.

31	2013	Duran	monkeys	Central America, South America	1	climate	NC, ND
32	2013	Forester	roseroot	North American Cordillera	1	climate	NC
33	2013	Gouveia	amphibians	global	1	climate	NC
34	2013	Hembry	phytophagous insects	Polynesia, French Polynesia	1	habitat	NC
35	2013	Hutter	anurans	Andes	1	climate	NC
36	2013	Khimoun	snapdragons	Europe	1	climate	ND
37	2013	Koch	ground lizard	Peru	1	climate	ND
38	2013	Lavergne	birds	Europe	3	climate, diet, habitat	NC, ND
39	2013	Martinez-Cabrera	geranium	South Africa	1	climate	ND
40	2013	Moen	frogs	Asia, Australia, South America	1	habitat	NC
41	2013	Palaoro	crayfish	USA, Mexico, Iberia	1	climate	NC
42	2013	Pellissier	arctic-alpine plants	European Alps, Norwegian Finnmark	1	climate	NC, ND
43	2013	Peterson	forest birds	global	1	climate	NC
44	2013	Razgour	bat	Europe	1	climate	NC
45	2013	Shipley	buntings	Southern United States	1	climate	NC
46	2013	Strubbe	birds	Europe	1	climate	NC
47	2013	Viljoen	sedges	Southern Hemisphere	1	climate	NC
48	2013	Wooten	salamander	North America	1	climate	ND
49	2013	Zengeya	Nile tilapia	Africa	1	climate	ND
50	2013	Zhu	stinkbug	Eastern Asia	1	climate	NC

Supp. Table 2_1 continued.

51	2013	Pau	C3 and C4 plants	Hawaii	2	climate, habitat	NC, ND
52	2012	Loera	Ephedra shrubs	North America, Mexico	1	climate	NC, ND
53	2012	Liu	C4 grasses	Africa, Australia, India	2	climate, habitat	NC
54	2012	Cicero	sage sparrow	Western North America	1	climate	ND
55	2012	Guggisberg	Canadian thistle	Europe, North America	1	climate	NC
56	2012	Litsios	damselfishes	global	2	climate, diet	NC, ND
57	2012	Larson	crayfish	global	1	climate	ND
58	2012	Temunovic	ash trees	Croatia	1	climate	ND
59	2012	Bett	doucs	Eastern Asia	1	climate	NC, ND
60	2011	Angetter	brown anole	Caribbean, Southern United States	1	climate	NC, ND
61	2011	Olalla-Tarraga	mammals, amphibians	global	1	climate	NC, ND
62	2011	Hulme	flowering plants	Washington DC, Oxfordshire	2	climate, time	NC, ND
63	2011	Couvreur	custard apple trees	African tropics	1	climate	NC
64	2011	Martin	orchard oriole	North America	1	climate	ND
65	2011	Bystriakova	scaly tree ferns	global	1	climate	NC, ND
66	2010	da Mata	fruit fly	tropical, sub-tropical regions	1	climate	ND
67	2010	Gallagher	plants	global	1	climate	ND
68	2010	Guillaumet	larks	France, Morocco	2	habitat, time	ND
69	2010	Hof	frogs	global	1	climate	NC
70	2010	Kozak	salamanderts	global	1	climate	ND

Supp. Table 2_1 continued.

71	2010	McCormack	jays	North America	1	climate	NC
72	2010	Medley	mosquito	SE Asia, New World, Europe	1	climate	ND
73	2009	Luxbacher	horned lizards	North America	1	climate	NC
74	2009	Murienne	cephalidiosus heteropterans	New Caledonia	1	climate	NC
75	2009	McNyset	freshwater fish	North America	1	climate	NC, ND
76	2009	Peterson	birds	Mexico	1	climate	NC
77	2009	Smith	angiosperms	global	1	climate	NC, ND
78	2009	Verbruggen	seaweed	tropical and subtropical coastal waters	1	climate	NC
79	2009	Vieites	salamanders	Holartic	1	climate	NC
80	2008	Banks	caribou, red deer	Europe	1	climate	NC
81	2008	Cordellier	limpets	NW Europe	1	climate	ND
82	2008	Eaton	blackbirds	New World	1	climate	NC, ND
83	2008	Peterson	thrush-like mourner	Neotropics	1	climate	NC
84	2008	Rodriguez-Sanchez	lauroid plants	Mediterranean basin, Macaronesian islands	1	climate	NC
85	2007	Diniz-Filho	salamander	New World	1	climate	NC
86	2007	Kozak	birds	New World	1	climate	NC, ND
87	2006	Knouft	anoles	Cuba	1	climate	NC, ND
88	2006	Kozak	salamanders	North America	1	climate	NC
89	2006	Lovette	wood warblers	North America	1	habitat	NC
90	2006	Wiens	Hylid frogs	New World	1	climate	NC

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Chapter 3

Supplemental Methods 3_1.

We assessed the accuracy of the copper models with embedded Thermochrons by equilibrating models at three biologically-relevant temperatures alongside a thermometer using a setup similar to Angilletta and Krochmal (2003). To this end we placed 28 models (as many as would fit) in a large incubator at the Concord Field Station, Harvard University in Bedford, MA and allowed them to acclimate over the course of an hour to three temperatures – 12, 23, and 32°C – after which temperature was recorded. On average, models deviated 0.13°C from the set incubator temperature, and the magnitude of deviation was consistently low across temperatures.

We measured the difference in equilibrium temperatures between copper models (T_e) and a live lizard (T_b) by exposing both to different temperatures, radiation, and convective conditions (reviewed in Dzialowski 2005). One live adult male *Anolis armouri* lizard and one copper lizard model were tethered to a cardboard panel using dental floss and a thermocouple (Type T, Omega) was inserted into the cloaca of each and secured using medical tape. The panel was exposed to different thermal conditions in fifteen minute intervals including: in the sun and shade on a sunny day (air temperature [T_a] = 31.1°C), outdoors on a breezy overcast day (T_a = 22°C), indoors in an air-conditioned room (T_a = 18.9°C), and indoors in a cold chamber (T_a = 4°C). To avoid thermal stress body temperatures (T_b) were not allowed to drop below 10°C or go above 35°C. T_e and T_b were automatically recorded every minute. The correlation between T_B and T_e was strong (0.889) and the coefficient of this relationship was close to 1 (coeff. =

1.03). This slope was offset by 0.86°C such that $T_B = 1.03(T_e) + 0.86$. Thus, to estimated T_b from T_e we corrected all copper model temperatures by adding 0.86°C.

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Supplemental Table 3_1. Coordinates and altitude (m) are given for each population sampled. Mountain chain for *Anolis cybotes* is given as W (Sierra de Baoruco, West) and E (Cordillera Central, East). Variable codes (bio 1 – bio 11) are as follows – bio 1: Mean Annual Temperature (MAT); bio 2: mean diurnal range; bio 3: isothermality; bio 4: temperature seasonality; bio 5: maximum temperature of the warmest month; bio 6: minimum temperature of the coldest month; bio 7: annual temperature range; bio 8: mean temperature of the wettest quarter; bio 9: mean temperature of the driest quarter; bio 10: mean temperature of the warmest quarter; bio 11: mean temperature of the coldest quarter. All values are in ˚C, except for bio 2 and bio 7.

Species	Coordinates	Alt.	bio 1	bio 2	bio 3	bio 4	bio 5	bio 6	bio 7	bio 8	bio 9	bio 10	bio 11
A. cybotes (W)	17.96, -71.19	45	26.0	9.9	0.76	1.03	32.2	19.3	12.9	26.8	24.6	27.2	24.5
A. cybotes (E)	18.43, -70.13	56	24.8	10.1	0.77	0.94	30.9	17.9	13.0	25.7	23.6	25.9	23.4
A. longitibialis	17.80, -71.45	105	25.8	9.9	0.73	1.10	32.3	18.9	13.4	26.9	24.5	27.2	24.3
A. whitemani	18.32, -71.57	411	24.3	12.3	0.76	1.13	31.9	15.9	16.0	25.3	22.7	25.6	22.7
A. strahmi	18.35, -71.63	454	22.3	12.9	0.77	1.18	30.0	13.3	16.7	23.3	20.6	23.6	20.6
A. marcanoi	18.55, -70.50	458	23.5	11.1	0.75	1.13	30.6	15.9	14.7	24.5	21.8	24.7	21.8
A. cybotes (E)	19.10, -70.61	690	23.1	10.8	0.76	1.01	29.6	15.5	14.1	24.1	21.6	24.2	21.6
A. cybotes (W)	18.06, -71.14	727	22.4	11.7	0.71	1.48	30.3	14.0	16.3	21.9	20.5	24.0	20.2
A. marcanoi	18.65, -70.54	879	19.6	13.3	0.77	1.20	27.7	10.5	17.2	20.6	18.1	20.9	17.8
A. cybotes (E)	18.86, -70.73	1390	18.0	12.4	0.77	1.12	25.4	9.3	16.1	19.0	16.4	19.2	16.4
A. cybotes (W)	18.18, -71.28	1395	17.2	13.0	0.76	1.29	25.1	8.2	16.9	17.7	15.5	18.6	15.3
A. shrevei	18.84, -70.70	1950	13.7	12.3	0.75	1.28	21.4	5.1	16.3	14.7	12.0	15.1	11.9
A. armouri	18.30, -71.70	2020	13.9	11.8	0.75	1.16	21.1	5.4	15.7	14.9	12.2	15.2	12.2
A. armouri	18.29, -71.71	2318	11.4	11.4	0.74	1.23	18.5	3.1	15.4	12.5	9.6	12.7	9.6
A. shrevei	18.73, -70.60	2450	10.1	11.7	0.74	1.35	17.5	1.8	15.7	11.4	8.4	11.6	8.2

Supplemental Table 3_2. Mean start temperature (˚C), rate of temperature change (˚C/min), and lizard body mass (g) are given for each population for CT$_{\text{min}}$ and CT$_{\text{max}}$ experiments. Values are mean ±1 SEM and sample sizes are given in parentheses. Only sample sizes that differ from the first column are given in other columns. Sample size is 16 for cooling rate in *A. cybotes* (Los Patos) and *A. cybotes* (Guayuyal).

Species	**Locality**	**CT$_{\text{min}}$**			**CT$_{\text{max}}$**		
		Start Temp. (ºC)	Cooling Rate (ºC/min)	Body Mass (g)	Start Temp. (ºC)	Heating Rate (ºC/min)	Body Mass (g)
A. cybotes (W)	Los Patos	26.7±0.9 (20)	0.99±0.01	7.42± 1.23 (19)	27.8±0.3	0.86±0.00	7.15±1.25
A. cybotes (E)	San Cristóbal	24.8±0.3 (16)	0.92±0.00	6.74±1.60	26.5±0.6	0.90±0.01	6.34±1.20
A. longitibialis	Jaragua	26.8±2.7 (18)	1.00±0.01	7.48±0.37	31.1±0.7	0.82±0.01	7.42±0.37
A. whitemani	Puerto Escondido	27.0±0.2 (15)	0.94±0.00	4.66±0.54	29.1±0.4	0.92±0.00	4.66±0.54
A. strahmi	Camino Aguacate	27.3±0.9 (6)	0.93±0.01	6.92±0.75	29.6±0.4	0.99±0.01	6.92±0.75
A. marcanoi	Ocoa	26.9±0.1 (9)	0.90±0.00	3.42±0.84	28.4±0.3	0.98±0.00	3.42±0.84
A. cybotes (E)	Jarabacoa	29.2±0.5 (18)	1.04±0.00	6.52±0.65	29.5±0.2	0.88±0.01	6.16±0.53
A. cybotes (W)	Guayullal	24.9±1.1 (18)	0.96±0.01	9.07± 1.37	28.2±0.3	0.86±0.01 (15)	8.45±1.62
A. marcanoi	La Horma	25.7±0.5 (16)	0.87±0.01	4.22±0.32	28.9±0.3	0.86±0.00	4.22±0.32
A. cybotes (E)	Constanza	24.6±0.5 (11)	0.97±0.01	4.95±1.11	24.8±0.3	0.98±0.01	4.65±1.15
A. cybotes (W)	La Hoz	27.7±0.3 (9)	0.92±0.00	6.84±2.25	30.8±0.1	0.86±0.00 (8)	6.02±3.24
A. shrevei	Valle Nuevo - Low	24.6±0.4 (9)	0.79±0.01	4.62±0.33	26.8±0.4	0.99±0.00	4.31±0.36
A. armouri	Loma de Toro - Low	24.4±0.8 (9)	0.94±0.02	5.82±1.29	26.8±1.1	0.98±0.00	5.23±1.81
A. armouri	Loma de Toro - High	29.7±0.2 (12)	0.93±0.00	6.43±1.86	-	-	-
A. shrevei	Valle Nuevo - High	26.6±0.8 (11)	0.84±0.01	4.79±0.07	28.5±0.1	0.76±0.01	4.46±0.07

Supplemental Table 3_3. Summary of results from linear regression assessing the relationship between mean population (A) critical thermal minimum, CT$_{min}$, and (B) critical thermal maximum, CT$_{max}$, and several variables - start temperature, body mass, and rate of temperature change. Regressions were weighted by the variance in each trait.

	Reg. Coeff.	d.f.	*t*	Pearson's *r*	*p*
A. CT$_{min}$					
Start Temperature	-0.21	13	-0.57	-0.107	0.578
Mass	-0.03	13	0.09	0.057	0.927
Cooling Rate	12.87	13	1.46	0.357	0.168
B. CT$_{max}$					
Start Temperature	-0.09	12	-0.84	-0.276	0.416
Mass	0.01	12	0.10	-0.107	0.924
Heating Rate	-0.97	12	-0.42	-0.134	0.682

Supplemental Table 3_4. Summary of loading, eigenvalues, and variance explained in the principal components (PC) analysis on the thermal variables (bio 1 – bio 11) for all populations sampled in this study. Loadings greater than 0.70 are highlighted in bold text.

Variable	**PC I**	**PC II**	**PC III**
MAT (bio 1)	**0.97**	-0.25	0.09
Mean Diurnal Range (bio 2)	-0.29	**0.95**	0.09
Isothermality (bio 3)	0.03	0.10	**0.99**
Temperature Seasonality (bio 4)	-0.32	0.57	**-0.74**
Maximum Temperature of Warmest Month (bio 5)	**0.99**	-0.13	0.05
Minimum Temperature of Coldest Month (bio 6)	**0.93**	-0.36	0.08
Temperature Annual Range (bio 7)	-0.32	**0.93**	-0.15
Mean Temperature of Wettest Quarter (bio 8)	**0.95**	-0.28	0.15
Mean Temperature of Driest Quarter (bio 9)	**0.96**	-0.26	0.11
Mean Temperature of Warmest Quarter (bio 10)	**0.97**	-0.24	0.06
Mean Temperature of Coldest Quarter (bio 11)	**0.96**	-0.27	0.12
Eigenvalue	8.1	1.6	1.3
Percent Variance Explained	73.2	14.2	12.2

Supplemental Table 3_5. PC scores for first three PC axes. Loadings are given in Supp. Table 3_4. MAT refers to 'mean annual temperature.'

Population	Locality	PC I – MAT	PC II – Range	PC III – Seasonality
A. cybotes (SB)	Los Patos	0.75	-1.18	0.37
A. cybotes (CC)	San Cristóbal	0.44	-1.18	1.05
A. longitibialis	Jaragua	0.87	-1.17	-0.95
A. whitemani	Puerto Escondido	0.98	1.00	0.44
A. strahmi	Camino Aguacate	0.64	1.50	0.83
A. marcanoi	Ocoa	0.56	-0.14	-0.08
A. cybotes (CC)	Jarabacoa	0.28	-0.61	0.61
A. cybotes (SB)	Guayullal	0.76	0.75	-2.50
A. marcanoi	La Horma	0.15	1.69	0.87
A. cybotes (CC)	Constanza	-0.47	0.66	1.01
A. cybotes (SB)	La Hoz	-0.40	1.30	0.25
A. shrevei	Valle Nuevo - Low	-1.29	0.42	-0.13
A. armouri	Loma de Toro - Low	-1.44	-0.18	0.16
A. armouri	Loma de Toro - High	-2.02	-0.64	-0.42
A. shrevei	Valle Nuevo - High	-2.16	-0.32	-0.69

Supplemental Table 3_6. Results of model fitting tests showing likelihood estimates and AIC$_C$ weights for the Brownian Motion (BM), Orenstein-Uhlenbeck (OU), and Early Burst (EB) models of evolution. Bold indicates the best-supported model.

	lnL			AIC$_C$ weight		
Trait	BM	OU	EB	BM	OU	EB
CT$_{min}$	-14.99	-14.38	-14.99	**0.98**	0.01	0.01
T$_b$	-13.20	-11.06	-13.20	**0.94**	0.05	0.01
CT$_{max}$	-5.86	-5.49	-5.86	**0.98**	0.01	0.01

Chapter 4

Supplemental Methods 4_1.

Body size – elevation relationships in the cybotes *clade (Hispaniola)*

We found a significant inverse body size cline pooling data from all species (Model 1; Supp. Table 4_1). As for the non-spatial analysis, this inverse cline arose due to interspecific differences. Even after accounting for elevation, species had significantly different intercepts, but not vice versa (Model 3; Supp. Table 4_1). Furthermore, slopes did not differ among species. As with non-spatial analysis, regressing each species' SVL against elevation revealed no significant intraspecific relationships (Supp. Table 4_2).

Body size – elevation relationships in the sagrei *clade (Cuba)*

On Cuba, we also found a significant inverse cline in body size with elevation after accounting for spatial autocorrelation (Supp. Table 4_1). We also found significant differences in species' slopes, indicating that intraspecific body size clines varied among species. Our individual species' regressions closely mirrored the non-spatial analysis, with the exception of *A. ahli*. This species slope was significant using a non-spatial model but non-significant using GLS (p = 0.052).

Sensitivity to random jittering of species' locales

We randomly jittered localities to avoid distances of zero separating species collected at the same location. In some cases, *p*-values were sensitive to this procedure, in particular for individual species regressions, though the balance of evidence still indicates significant relationships in these species (Supp. Tables 4_1, 4_2; Supp. Fig. 4_2, 4_3)

Supp. Table 4_1. Significance tests of four GLS models fit to the *cybotes* clade on Hispaniola and *sagrei* clade on Cuba to account for spatial autocorrelation while evaluating the relationship between snout-vent length and elevation (ln(elevation+0.5)). Model 1 assumed a common slope and intercept among species, Model 2 constrained slopes to zero and allowed intercepts to vary (i.e., tested for mean differences among species), Model 3 assumed a common slope and varying intercepts, and Model 4 allowed slopes and intercepts to vary. All significant tests were performed using marginal sums of squares. Medians of 100 fits to each model are presented, each with a unique, small random jitter added to coordinate to avoid distances of zero between localities. Spatial autocorrelation structure was chosen using AIC_c. Significant relationships are in bold.

cybotes clade, Hispaniola

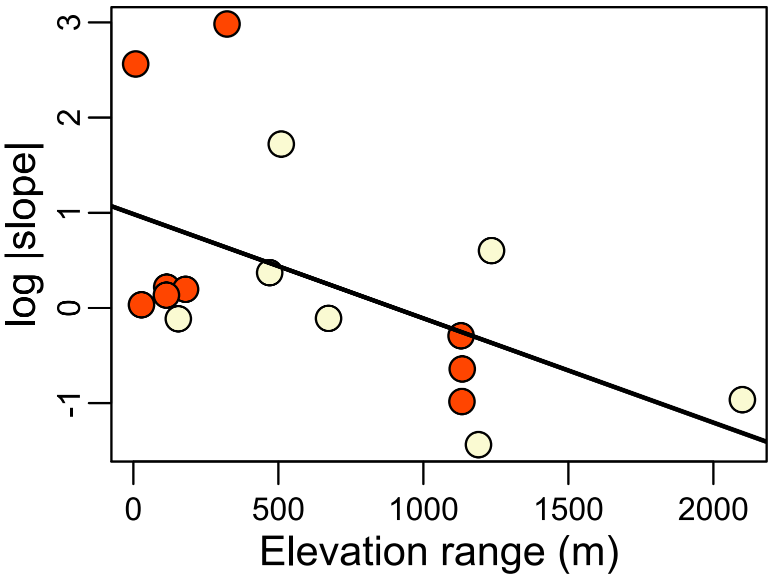
Model Num	Spatial correlation structure	Common slope			Intercept differences			Slope differences		
		F	df	P	F	df	P	F	df	P
1	Exponential	59.4	1, 433	$< 1\times10^{-13}$	-	-	-	-	-	-
2	Exponential	-	-	-	76.9	6, 428	$< 1\times10^{-15}$	-	-	-
3	Gaussian	1.0	1, 427	0.32	46.8	6, 427	$< 1\times10^{-15}$	-	-	-
4	Exponential	-	-	-	-	-	-	1.57	6,421	0.15

sagrei clade, Cuba

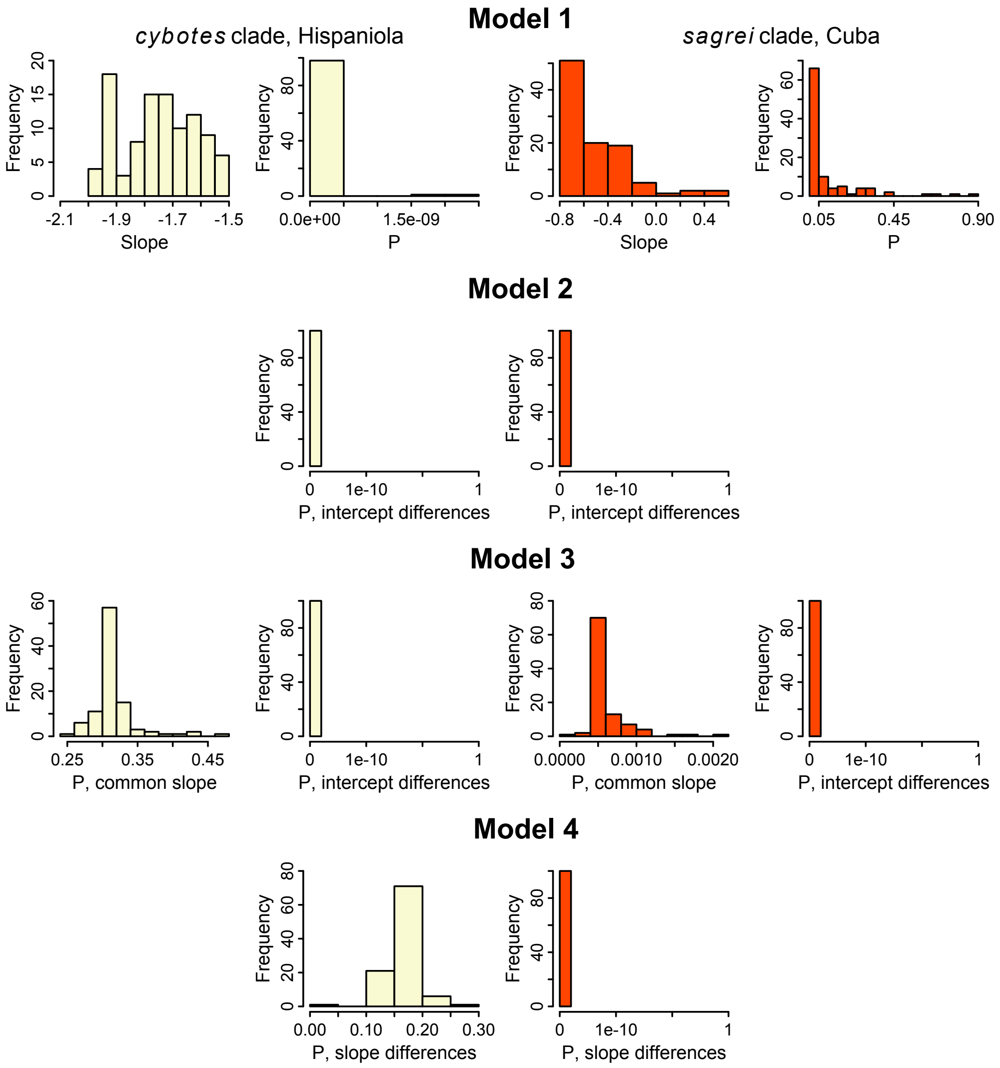
Model Num	Spatial correlation structure	Common slope			Intercept differences			Slope differences		
		F	df	P	F	df	P	F	df	P
1	Exponential	8.4	1, 669	0.0038	-	-	-	-	-	-
2	Spherical	-	-	-	122.3	8, 662	$< 1\times10^{-15}$	-	-	-
3	Gaussian	11.9	1, 661	5.1×10^{-4}	131.5	8, 661	$< 1\times10^{-15}$	-	-	-
4	Spherical	-	-	-	-	-	-	15.6	8, 653	$< 1\times10^{-15}$

Supp. Table 4_2. GLS models showing the body size-elevation relationships for individual species. The results for the GLS analysis closely mirrored those from the non-spatial analysis, save for the relationship for *A. ahli*, which is significant in the GLM analysis (Table 4). For the GLS models, autocorrelation structure was chosen using AICc. 100 repetitions were performed for each species, with a different random jitter added to localities each time to avoid neighbor distances of zero. The model with the median slope is presented below. As P-values were sensitive to jittering in some cases, the final column gives the number of these 100 models that had a slope with a P-value < 0.05.

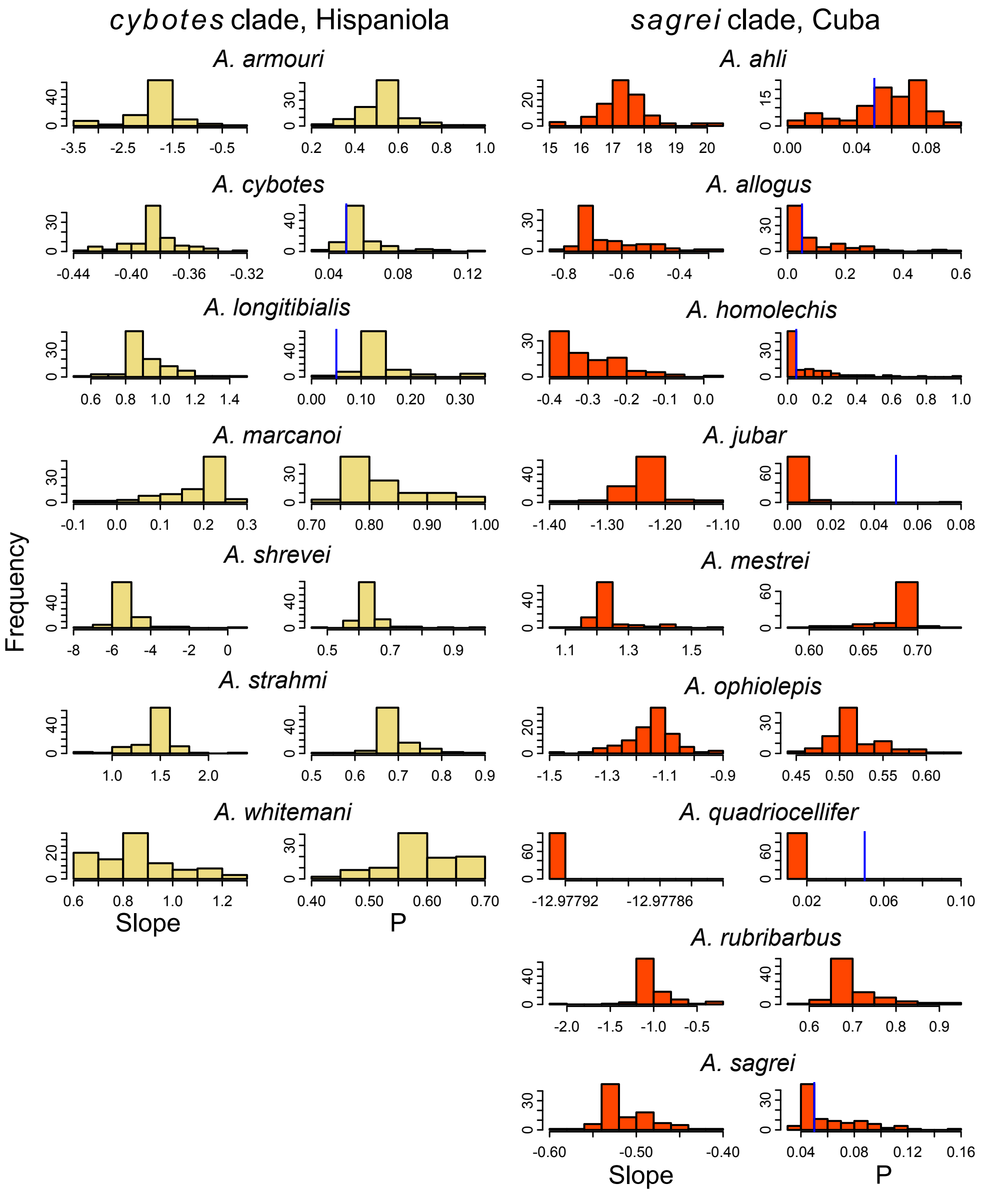
Species	**Clade**	**Median Slope (s.e).**	**t**	**d.f.**	***p***	**Cor. Struct.**	**# *p* < 0.05**
		Hispaniola					
armouri	*cybotes*	-1.83 (2.69)	-0.68	27	0.50	Gaussian	0
cybotes	*cybotes*	-0.38 (0.20)	-1.93	298	0.055	Spherical	14
longitibialis	*cybotes*	0.89 (0.56)	1.58	24	0.13	Spherical	2
marcanoi	*cybotes*	0.22 (0.84)	0.26	38	0.80	Gaussian	0
shrevei	*cybotes*	-5.59 (10.69)	-0.52	15	0.61	Exponential	0
strahmi	*cybotes*	1.45 (3.59)	0.40	14	0.69	Gaussian	0
whitemani	*cybotes*	0.90 (1.46)	0.61	5	0.57	Exponential	0
		Cuba					
ahli	*sagrei*	17.34 (7.41)	2.34	7	0.052	Exponential	28
allogus	*sagrei*	-0.70 (0.34)	-2.09	94	0.039	Gaussian	53
homolechis	*sagrei*	-0.33 (0.16)	-2.01	266	0.046	Exponential	52
jubar	*sagrei*	-1.25 (0.39)	-3.17	43	0.0028	Gaussian	99
mestrei	*sagrei*	1.22 (2.88)	0.42	11	0.68	Exponential	0
ophiolepis	*sagrei*	-1.44 (1.65)	-0.69	11	0.50	Exponential	0
quadriocellifer	*sagrei*	-12.98 (2.61)	-4.96	3	0.016	Gaussian	100
rubribarbus	*sagrei*	-1.03 (2.45)	-0.42	14	0.68	Gaussian	0
sagrei	*sagrei*	-0.52 (0.26)	-2.01	204	0.046	Exponential	50



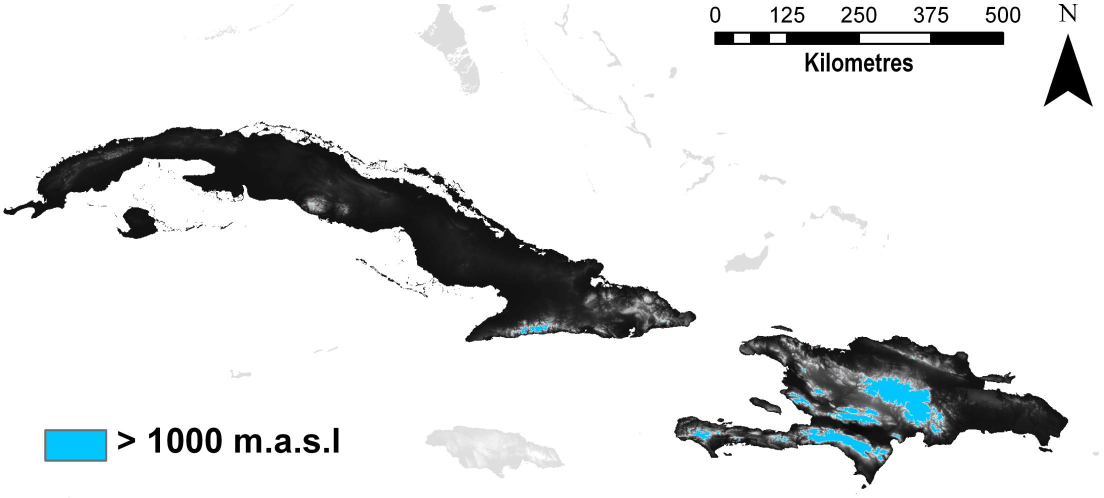
Supp. Fig. 4_1. The relationship between the absolute slope of the body size – elevation regression and the elevation range of sixteen species of anole from the *cybotes* clade on Hispaniola (light yellow) and the *sagrei* clade on Cuba (orange-red). Absolute slopes were log transformed to reduce skew of residuals.



Supp. Fig. 4_2. *p*-values for coefficients of GLS models linking body size and elevation (ln(elevation+0.5)), accounting for spatial autocorrelation. Model 1 assumed a common slope and intercept among species, Model 2 constrained slopes to zero and allowed intercepts to vary (i.e., tested for mean differences among species), Model 3 assumed a common slope and varying intercepts, and Model 4 allowed slopes and intercepts to vary. P-values were based on marginal sums of squares. Results of 100 fits to each model are presented, each with a unique, small random jitter added to coordinate to avoid distances of zero between localities.



Supp. Fig 4_3. Slope and *p*-values for GLS regressions of snout-vent length on ln(elevation+0.5) for individual species, accounting for spatial autocorrelation. Results of 100 regressions for each species are presented, each with a unique, small random jitter added to coordinate to avoid distances of zero between localities. The *cybotes* clade (Hispaniola) results are in pale yellow and *sagrei* clade (Cuba) results in orange-red.



Supp. Fig. 4_4. Highland areas of Hispaniola and Cuba (blue). Highland areas were defined as those above 1,000 meters above sea level (m.a.s.l).

Chapter 5

Supplemental Methods 5_1.

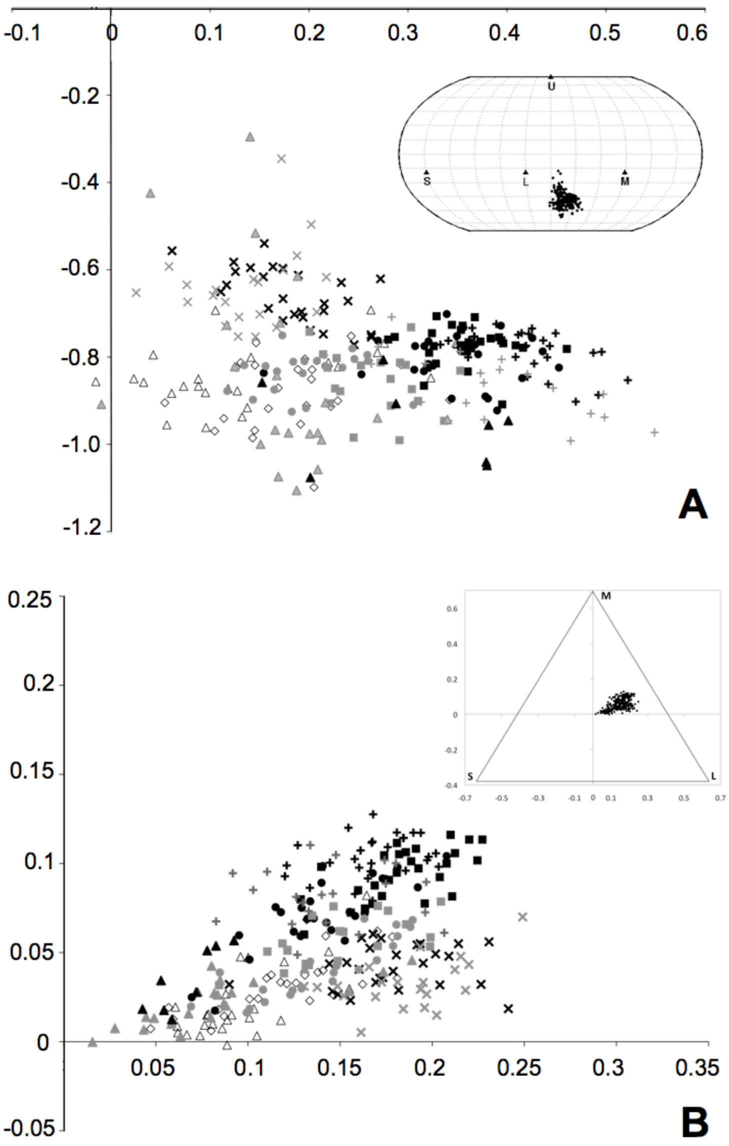
Microsatellite testing and selection

We identified over 500 tri- and tetra- nucleotide repeats from the 3072 genomic library sequence reads produced by the Broad Institute of MIT and Harvard and designed primers for these loci using MSATCommander (Faircloth 2008). We screened 192 of these loci on a panel of eight individuals from populations of *A. m. marmoratus* on the adjacent island of Basse Terre and identified ten loci that amplified consistently and produced clear genotypes. Of those ten, seven were amplifiable and easily scored in our sample from Grande Terre. To genotype microsatellite loci, one member of each primer pair contained a tail complementary to the M13 Reverse primer or the CAG primer (see Faircloth 2008). PCR products were fluorescently labeled with either an M13R-FAM or CAG-NED fluorescently labeled primer. We optimized results by amplifying with unlabeled primers first and then using those templates in a labeling reaction with the fluorescently labeled M13R or CAG 10x primers relative to the reverse primer. Fragments were sized using a Naurox standard (DeWoody et al. 2004) on an ABI-3100 sequencer and visually inspected using GeneMapper v3.7 (Applied Biosystems).

Works Cited

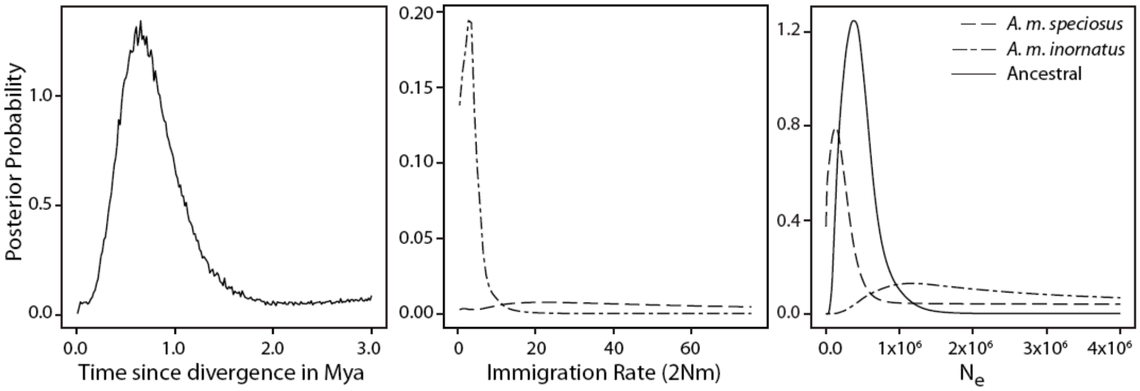
DeWoody, J.A., Schupp, J., Kenefic, L., Busch, J., Murfitt, L., Keim, P. 2004. A universal method for producing ROX-labeled size standards suitable for automated genotyping. *Biotechniques* 37:348—352.

Faircloth, B.C. 2008. MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8:92—94.



Supp. Fig. 5_1. (A) Detail of Robinson projection (Endler et al. 2005; Stoddard and Prum 2008) of lizard and visual background color components showing hue longitude on the x-axis and hue latitude on the y-axis calculated using the cone sensitivities of *Anolis cristatellus* (Loew et al. 2002). Inset shows position of all points in Robinson projection of complete tetrahedral color space. Δ = reflectance of visual background (vegetation and leaf-litter). ◊ = dorsolateral reflectance of females. Other symbols are reflectance from

Supp. Fig. 5_1 continued: adult males. Grey symbols represent *A. m. inornatus*, black symbols represent *A. m. speciosus*: ▲= eye ring; + = lateral tail; l = dorsolateral head (temporal region); n = dorsolateral body (shoulder region); 5 = center of the dewlap. (B) Plots of lizard color components and visual background projected on the S, M, L face of the color space tetrahedron (looking down along the UV axis). The scale and orientation of the S, M, L color space on the x-y plot is shown in the inset. Symbols are the same as above.



Supp. Fig. 5_2. Posterior probability distributions for the demographic parameters of *Anolis marmoratus* estimated from 1,731,863 genealogies generated by six independent MCMC runs of IMa2 (see text for model parameters). Left: Time since divergence, *t*, between both populations of *A. marmoratus*. Center: The amount of gene flow, *m*, from *A. m. inornatus* into *A. m. speciosus* (broken hash) and the amount of gene flow, *m*, from *A. m. speciosus* into *A. m. inornatus* (continuous hash). Right: The effective population size (N_e) of for the ancestral (solid) and current populations of *A. m. speciosus* (continuous hash) and *A. m. inornatus* (broken hash).