

Physiological Variation among Invasive Populations of the Brown Anole (*Anolis sagrei*)^{*}

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ABSTRACT

Invasive species often encounter novel climatic conditions when they spread outside of their native ranges. Invading populations can respond to novel conditions by acclimation or adaptation of physiological capacities, which may facilitate their spread. We investigated differences in physiological traits among three populations of an invasive lizard, the brown anole (*Anolis sagrei*), along the latitudinal extent of its invasion in the southeastern United States. We predicted latitudinal clines for most traits based on models of adaptation to climate. Consistent with the latitudinal cline in temperature and moisture, the mean critical thermal minimum and the mean rate of water loss were lowest for lizards in the northern population. Furthermore, these traits acclimated to either temperature or humidity in a direction consistent with adaptive phenotypic plasticity. By contrast, metabolic rates varied among populations but did not conform to our prediction based on a latitudinal cline in temperature. Critical thermal maxima, endurance, and sprint speeds were similar among populations. Despite the idea that tropical lizards have limited capacity for acclimation, we found variation among invasive populations of brown anoles, which could have partially resulted from acclimation. This physiological variation within the invasive range raises questions about the roles of plasticity and adaptation in the success of the invasion.

Introduction

To successfully invade a new region, a species must colonize, establish, and spread (Sakai et al. 2001). To do so, invading genotypes must survive and reproduce, often under novel climatic conditions (e.g., Broennimann et al. 2007; Kolbe et al. 2012). For example, as cane toads (*Bufo marinus*) expanded their invasive distribution south in Australia, they experienced lower and more variable temperatures (Urban et al. 2007; Kearney et al. 2008). Thermal tolerances reflected acclimation to these novel conditions, which likely conferred higher survival and facilitated the range expansion (Kolbe et al. 2010). If invading populations can adjust their phenotypes to match local conditions, either through plasticity or adaptation, the likelihood of a successful invasion increases.

Although the importance of physiological tolerance surely varies at different stages of the invasion, invading populations tend to encounter novel climates when spreading beyond the initial point of colonization. These climates can stimulate acclimation or adaptation of physiological capacities. Natural selection throughout the invasive range could lead to local adaptation of traits that facilitate the spread of the species. For example, the wing sizes of fruit flies (*Drosophila subobscura*) diverged genetically along latitudinal gradients in North and South America following invasions from Europe (Huey et al. 2000, 2005). In contrast to adaptation, genetic differences within the invasive range can also result from sequential introductions from different areas in the native range. Introductions from multiple sources occur commonly during invasions (Dlugosch and Parker 2008); for instance, seven of the eight species of *Anolis* lizards that were introduced to Florida came from multiple sources (Kolbe et al. 2007a). Climatic differences can also trigger acclimation of physiological traits, with or without further adaptation of the reaction norm for these traits (e.g., Kolbe et al. 2012). The first step toward learning how acclimation and adaptation contribute to successful invasions is to quantify physiological variation throughout the invasive range. If populations show phenotypic variation across an environmental gradient, one can pursue additional studies designed to quantify acclimation or adaptation.

We used the invasion of the southeastern United States by the brown anole (*Anolis sagrei*) to study variation in physiological traits among invasive populations. This system confers several advantages. First, the invasive range spans a broad latitudinal extent, from southern Florida to Georgia (~24.5°N to 33°N). Second, we have some information about the physiological ecology of lizards that occur in this region (e.g., Wilson and Echternacht 1987; Angilletta 2001a, 2001b), including the native green anole (*Anolis carolinensis*). Finally, the history of

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the invasion has been inferred from molecular genetics; populations in the United States come from at least seven sources within Cuba (Kolbe et al. 2004). Currently, brown anoles occur throughout Florida with extensive admixture among the different sources (Kolbe et al. 2008). By contrast, disjunct populations in Georgia occur primarily along interstate highways (Campbell 1996). The populations that we sampled span the latitudinal extent of the invasion and have distinct histories of colonization: a population in Miami, Florida (25.5°N), which came from mostly western Cuba and was established in the 1950s; a population in Orlando, Florida (28.3°N), which came from sources in western, central, and eastern Cuba beginning in the 1970s; and a population in Tifton, Georgia (31.5°N), which came from an unknown source in the 1990s (Bell 1953; Godley et al. 1981; Campbell and Hammontree 1995; Campbell 1996; Kolbe et al. 2007b). Despite the uncertain origin of the Tifton population, molecular genetics indicate that a population in Valdosta, Georgia, about 75 km south of Tifton, was derived from sources in western and central Cuba (S. Pau and C. Infante, personal communication).

We quantified physiological variation among the three invasive populations. In doing so, we expected to find latitudinal clines in traits that were predicted from models of adaptation to climate (Angilletta 2009). Since minimal air temperatures vary latitudinally more than do maximal air temperatures (fig. 1), we expected that lizards from the northernmost population (Tifton) would tolerate the lowest body temperatures; however, we did not expect a strong cline in heat tolerance among populations. A cline in cold tolerance has been documented in the native congener, *Anolis carolinensis* (Wilson and Echternacht 1987). Since lizards in northern populations experience the lowest relative humidities (fig. 1), we predicted greater resistance to water loss, similar to patterns observed in other species of *Anolis* (Hertz et al. 1979; Hillman et al. 1979; Dmi'el et al. 1997). The overall lower temperatures in northern populations predicted better performance (i.e., sprint speed and endurance) at low temperatures for these populations compared to southern ones. Finally, we thought that metabolic rate of anoles from colder populations would be greater, which has been observed for other species that remain active during winter (e.g., Tsuji 1988a). When latitudinal variation was observed, we conducted additional experiments to quantify the potential for acclimation within populations. Based on these studies, we inferred whether adaptation or plasticity might have played a role in the spread of the brown anole in the southeastern United States.

Material and Methods

Collection and Care of Lizards

In May 2006, we collected juveniles and adults of both sexes from each population. Lizards were transported to the laboratory at Indiana State University, where they were housed individually in plastic terraria. Each terrarium was lined with artificial grass and contained a plastic plant and perches for climbing. Terraria were placed in incubators that maintained a temperature of 29°C and a 12L : 12D photoperiod. Lizards from

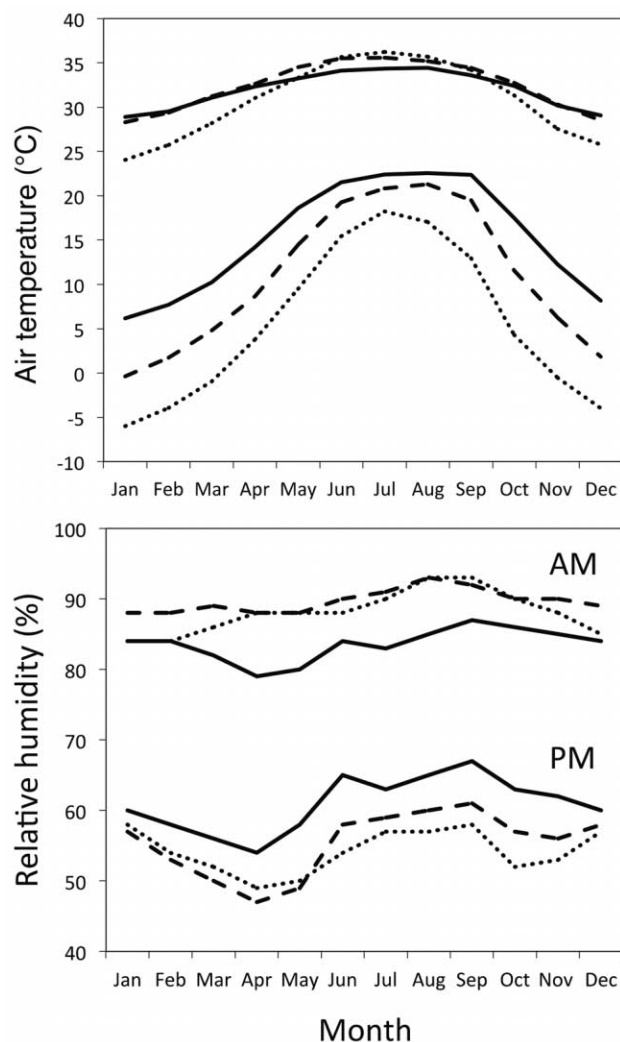


Figure 1. Analysis of climatic data confirmed expected patterns of temperature and relative humidity among populations. *Top*: mean daily maximum of air temperature (upper lines) differed modestly among populations, with Tifton (dotted line) having lower values than Orlando (dashed line) and Miami (solid line) only from late fall to early spring. By contrast, the mean daily minimum of air temperature (lower lines) varied markedly among sites, with Tifton having the lowest minimum temperatures throughout the year. Monthly extreme daily air temperatures for 1970–2012 were obtained from the National Climatic Data Center (stations 12839, 12815, and 98703). *Bottom*: relative humidities were consistently higher in the afternoon (PM) in Miami as well as less variable during the day (AM and PM) in Miami compared to other sites. Mean monthly relative humidities for 1964–2003 were obtained from the National Climate Data Center from stations in Miami, Orlando, and Macon, Georgia, which is about 150 km north of Tifton.

each population were dispersed throughout the incubators, and terraria were rotated daily to minimize the effects of potential gradients in temperature, light, and humidity. Experiments began within a few days of captivity and lasted for 3 wk. In October 2008, we obtained adults of both sexes from Miami and west of Orlando. Lizards were housed in the laboratory at

the University of California, Berkeley, under conditions similar to those described above, except that temperature was based on an acclimation treatment (see “Acclimation of Critical Thermal Minima”). In October 2011, we collected adults from Miami and housed them in the laboratory at the University of Rhode Island under conditions similar to those described above, except that relative humidity was based on an acclimation treatment (see “Acclimation of Critical Thermal Minima”). The Institutional Animal Care and Use Committees of Indiana State University, the University of California, Berkeley, and the University of Rhode Island approved the procedures in this study.

Climatic Variation

To compare the climate space occupied by brown anoles in their native range (Cuba) and invasive range (Florida and Georgia), we used georeferenced locations for museum specimens, which we obtained from HerpNet (<http://www.herpnet.org/>). We also used locations determined from fieldwork for a total of 369 and 397 locations in the native and invasive ranges, respectively (J. J. Kolbe, unpublished data). We evaluated 19 variables based on temperature and precipitation: BIO 1–19 from the WORLDCLIM 1.4 data set (Hijmans et al. 2005). These data layers represent annual trends, seasonality, and extremes of temperature and precipitation. Using data extracted at each locality, we generated a Pearson product correlation matrix to identify and remove highly correlated variables ($r \geq 0.85$). This yielded nine variables for our analysis: annual mean temperature (BIO 1); mean diurnal range, or mean of monthly values for maximum temperature minus minimum temperature (BIO 2); maximum temperature of the warmest month (BIO 5); mean temperature of the wettest quarter (BIO 8); mean temperature of the warmest quarter (BIO 10); annual precipitation (BIO 12); precipitation of the wettest month (BIO 13); precipitation of the driest month (BIO 14); and precipitation of the warmest quarter (BIO 18). We used a discriminant function analysis to predict the grouping of lizard locations (native vs. invasive range) based on these bioclimatic data.

Thermal Tolerances

To assess thermal tolerances, we measured critical thermal maxima and critical thermal minima; these common indexes are defined as the upper and lower temperatures at which an animal loses its ability to right itself when placed on its back (Lutterschmidt and Hutchison 1997). For both procedures, we tested adult lizards in sets of three, one from each population to minimize temporal effects on tolerance. Before measuring critical thermal maxima, lizards were kept at 37°C for 1 h. We then held each lizard near a 60-W infrared bulb (Pearlco, Ram Network, Reseda, CA) to raise the lizard’s temperature by $\leq 1^\circ\text{C}$ per minute. We tested the righting response of each lizard by flipping it on its back and stimulating its venter with a small probe. If a lizard righted itself within 30 s, it was heated further and retested when showing signs of lethargy. If the lizard did

not right itself, its body temperature was measured with a fine-gauge thermocouple or a cloacal thermometer (Miller and Weber, Queens, New York) and recorded as its critical thermal maximum. We determined critical thermal minima in a similar manner, except that lizards were cooled in small plastic containers set on a tray of ice. All lizards survived both procedures.

We used generalized linear modeling to describe the effects of population, sex/stage (adult male, adult female, or juvenile), and body mass on critical thermal limits. Following Zuur and colleagues (2009), we started by fitting a model with all possible main effects and interactions. We used the Akaike Information Criterion (AIC; Burnham and Anderson 2002) to compare the fit of a model with a Gaussian distribution of error to that of a model with a gamma distribution. For both critical thermal maximum and minimum, the Gaussian distribution was a better choice. Once we had determined the best structure for the error term, we progressively simplified each model by dropping the highest-order term and seeing whether the simpler model was more likely (as indicated by a lower value of AIC). Analyses were completed using the R statistical package (ver. 2.13.1; R Development Core Team 2011).

Water Loss

We measured rates of water loss by calculating the change in body masses of lizards held at a constant temperature (29.4°C) and relative humidity (31%). Measurements were taken in a walk-in environmental chamber. We placed lizards individually in plastic mesh bags and suspended each one on a hook inside of a hood, which kept them in the dark when not being weighed. This procedure not only limited water loss due to conduction and convection but also greatly reduced activity. After taking the initial mass, we weighed lizards every 20 min during a period of 140 min. Masses were recorded to the nearest 0.1 mg using an electronic balance. In rare cases, lizards defecated during the experiment; data from these periods were omitted from our analyses.

We used general linear mixed modeling to describe the effects of time, population, and sex/age class (males, females, and juveniles) on body mass during dehydration. A model with a gamma distribution of error provided a much better fit than did one with a Gaussian distribution ($\Delta\text{AIC} = 39.8$). Using the gamma distribution as an error structure, we progressively simplified the model by dropping the highest-order terms and using AIC to infer the most likely model. Analyses were completed using the R statistical package (version 2.13.1; R Development Core Team 2011).

Metabolic Rate

We measured the rate of oxygen consumption by each lizard at four temperatures: 25°, 29°, 33°, and 37°C. Measurements were made at one temperature per day, and the order of temperatures was determined randomly. Thus, the following procedure was repeated daily until measurements had been performed at all temperatures. At 1700 hours, lizards were weighed

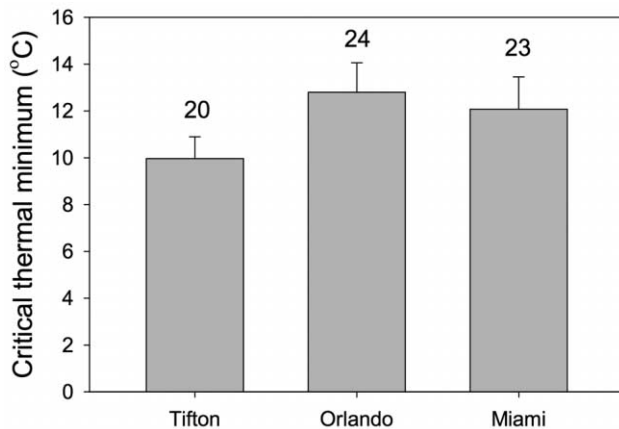


Figure 2. Brown anoles from Tifton tolerated lower temperatures than did anoles from Orlando or Miami. Error bars denote standard deviations, and numbers indicate sample sizes.

to the nearest 0.1 mg and sealed in respirometry chambers (250 mL). The chambers were placed in a dark incubator (model KB 115, Brinkmann). To ensure that body temperatures equaled the air temperature of the incubator, chambers were placed in the incubators at least 1 h before measurements. Recordings always commenced at 2100 hours. Initially, all chambers were flushed sequentially with atmospheric air purged of water and carbon dioxide by a gas generator (model 75-45, Parker Balston). Each chamber was flushed again 2–3 h after the initial flush. At each flush, air from the chambers was passed through a column of magnesium perchlorate, a CO₂ analyzer (model LI-6251, Li-Cor, Lincoln, NE), an oxygen analyzer (model FC-1, Sable Systems, Henderson, NV), and a mass-flow meter (v1.0, Sable Systems). In between the CO₂ analyzer and the O₂ analyzer, CO₂ was removed from the airstream by soda lime and ascarite. Before the study, the mass-flow meter was calibrated using a mass-flow controller valve (Sidetrak, Sierra Instruments, Monterey, CA) connected to a mass flow controller electronic unit (v1.0, Sable Systems). A computer controlled the sequential flushing of chambers to ensure that each chamber was sealed for a precise duration between recordings.

Using records of oxygen concentration and flow rate, we calculated the total volume of oxygen consumed while the chamber was sealed. Each recording was analyzed by a computer program (CONVOL, Sable Systems) to generate an hourly rate of oxygen consumption (mL h⁻¹). We used general linear mixed modeling to describe the effects of population, sex/stage, mass, and temperature on the rate of oxygen consumption. To avoid pseudoreplication, the identity of the lizard was included as a random factor. Metabolic rate was modeled in the same way that we modeled water loss (see above).

Sprint Speed

We measured sprint speeds of adult lizards at seven temperatures (15°, 20°, 25°, 29°, 32°, 36°, and 39°C). These measure-

ments were made on a racetrack housed in a walk-in environmental chamber. The 1.5-m track was positioned at an angle of 45°, as used in previous studies on anoles (Losos and Sinervo 1989). The chamber was set at the desired temperature and a relative humidity of 60%. About 60–90 min before each measurement, a lizard was placed in the chamber to enable its temperature to approach that of the air in the chamber. Just before each run, we weighed the lizard and measured its temperature with a cloacal thermometer to ensure that it has reached the desired temperature. One of us placed the lizard at the base of the track and then chased it up the length of the track by hand. A second person recorded the duration of the run with a stopwatch.

Our experimental design controlled for potentially confounding effects of time. We measured speed at different temperatures on consecutive days and randomized the order of lizards from each population to avoid confounding effects of population and time of day. At the end of each day, lizards were offered food and water at the end of each trial. We used the fastest of the three trials at each temperature as an estimate of maximal speed. Individuals that did not perform at one or more temperatures were omitted from our analyses.

We used general linear mixed modeling to describe the effects of population, sex, mass, and temperature on sprint speed. To avoid pseudoreplication, the identity of the lizard was included as a random factor. Sprint speed was modeled in the same way that we modeled water loss (see above).

Locomotor Endurance

We measured the endurance of adult lizards by forcing them to run continuously in a circular arena (Bennett 1980). The arena measured 1.8 m in diameter and contained a substrate

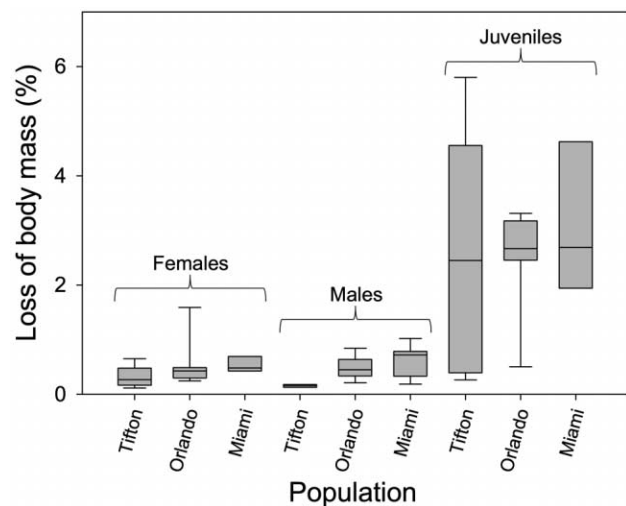


Figure 3. Brown anoles from Tifton lost water at slower rates than did anoles from Orlando or Miami. Boxes and whiskers denote the central 50% and 90% of values, respectively.

Table 1: Inferential statistics for the most likely generalized linear mixed model of mass loss during dehydration

Effect	Effect df	Error df	F	P
Initial mass	1	84	39.29	<.0001
Sex	2	82	15.62	<.0001
Population	2	80	7.57	.0010
Initial mass × time	2	78	3.92	.0239

Note. This model fitted the data substantially better than the second-best model (ΔAIC [Akaike Information Criterion] = 4.2), which included the interaction between initial mass and sex.

of sand. This arena was housed in a walk-in environmental chamber that maintained a temperature of 33°C and a relative humidity of 60%. Each lizard was placed in the chamber 60 min before measuring endurance to ensure that it reached the desired body temperature. Then, we released the lizard into the arena and chased it with a pole to stimulate movement. If a lizard attempted to climb the wall of the arena, it was gently nudged down and encouraged to continue running. When lizards slowed or paused, we flipped them over to test their righting response. If a lizard failed to right itself, the time was marked. Endurance was estimated as the time from the start to the end of the trial. Each lizard was run only once.

We used generalized least squares to estimate the effects of population, sex, and snout-vent length on endurance. This procedure enabled us to fit a separate error term for each of the three populations. A model with separate error terms was more than three times as likely to be the best model than one with a single error term ($\Delta\text{AIC} = 2.6$). Models were fit using the “nlme” library (Pinheiro et al. 2011) of the R statistical package (R Development Core Team 2011).

Acclimation of Critical Thermal Minima

To determine the potential to acclimate low-temperature tolerance, we measured critical thermal minima of lizards repeatedly while exposing them to one of two thermal regimes for 18 wk. On average, lizards under warm conditions experienced minimal and maximal daily temperatures of 23.8° and 32.7°C (mean = 28.9°C). Lizards under cool conditions experienced minimal and maximal daily temperatures of 17.3° and 21.7°C (mean = 19.6°C). The treatments simulated thermal conditions in summer and winter, respectively (Kolbe et al. 2012). Lizards from Orlando and Miami were randomly assigned to treatments, such that 12 lizards from each population were in each thermal condition. We measured the critical thermal minimum of each lizard at 0, 2, 6, 10, 14, and 18 wk of exposure. Three lizards from the cool treatment died before the end of the experiment but not during assays of critical thermal minima.

We used general linear mixed modeling to describe the effects of population, sex, mass, time (week), and acclimation treatment on the critical thermal minimum. The identity of each lizard was included as a random factor. A correlation structure was added to describe variation in the critical thermal mini-

um among weeks within each individual. Models were fit using the “nlme” library in R (Pinheiro et al. 2011; R Development Core Team 2011).

Acclimation of Water Loss

To determine the potential to acclimate rates of water loss, we exposed lizards from Miami to one of two relative humidities for 2 wk and then estimated rates of water loss. Before the experiment, lizards were kept at a mean temperature of 21.0°C and a relative humidity of 60% for 5 wk. We randomly assigned 29 lizards to either a high or low treatment of relative humidity. In contrast to previous experiments, our experiment comprised treatments that mimic diel variation in relative humidity (fig. 1): the high-humidity treatment averaged 60% (minimum and maximum daily humidity averaged 42% and 78%, respectively) and the low-humidity treatment averaged 37% (minimum and maximum daily humidity averaged 26% and 52%, respectively). Temperature averaged 22.8°C in both treatments. We estimated rates of water loss before and after the 2-wk experiment. This procedure was the same as our comparative study of water loss (see “Water Loss”), except that lizards were held at a temperature of 28.5°C and a relative humidity of 23% and were weighed after 0, 1, 2, 4, and 6 h of exposure.

We used general linear mixed modeling to describe the effects of mass, treatment (high or low humidity), trial (before or after), and time (0–6 h) on body mass during dehydration. The identity of each lizard was included as a random factor. Model selection was performed as described above (see “Water Loss”).

Table 2: Inferential statistics for the most likely general linear mixed model of metabolic rate

Effect	Effect df	Error df	F	P
Intercept	1	233	.167	.683
Mass	1	85	1.206	.305
Sex/stage	2	233	15.806	.0001
Population	2	85	.376	.688
Temperature	3	233	1.501	.215
Mass × population	2	233	29.905	<.0001
Mass × temperature	3	233	24.343	<.0001
Population × temperature	6	233	.919	.482
Mass × sex/stage	2	233	2.524	.082
Sex/stage × temperature	6	233	2.205	.043
Mass × population × temperature	6	233	4.854	.0001

Note. The standard deviation of the random effect of individual equaled 0.07 mL O₂ h⁻¹. This model fitted the data substantially better than the second-best model (ΔAIC [Akaike Information Criterion] = 2.0), which excluded the interaction between sex/stage and temperature.

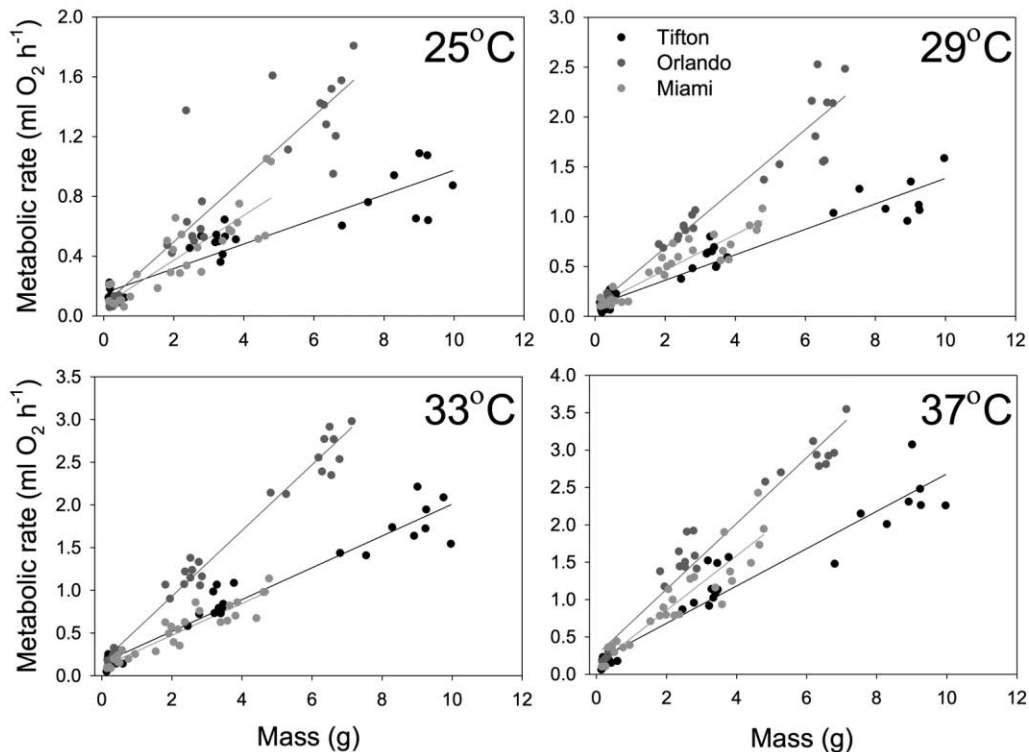


Figure 4. At all four temperatures, brown anoles from Tifton had the lowest metabolic rates, whereas those from Orlando had the highest rate. Note that the scale for metabolic rate varies among temperatures. A color version of this figure is available online.

Results

Climatic Variation

The discriminant function analysis correctly classified all locations as being within either the invasive or native range. Compared to the native range, locations in the invasive range had lower annual mean temperatures (BIO 1), higher mean diurnal temperature ranges (BIO 2), and intermediate annual precipitation (BIO 12). Therefore, brown anoles experienced novel abiotic conditions following their invasion of the southeastern United States (see Angetter et al. 2011).

Thermal Tolerances

The most likely model of the critical thermal minimum included only the effect of population (deviance = 92.19, $F_{2,64} = 31.12$, $P < 0.0001$). Anoles from Tifton tolerated lower temperatures than did anoles from Orlando or Miami (fig. 2). The second-best model ($\Delta\text{AIC} = 1.2$) also included a weak, negative effect of body mass ($-0.05^\circ\text{C g}^{-1}$; $P = 0.42$) as an independent variable. By contrast, brown anoles of both sexes from all populations had similar critical thermal maxima, and thus the most likely model excluded these factors; the grand mean for the three populations was 43.0°C (SE = 0.23°C , $n = 57$). This model fit the data substantially better than the second-best model ($\Delta\text{AIC} = 3.0$), which included an effect of sex ($P = 0.50$).

Water Loss

Anoles from Miami and Orlando lost water more rapidly than did anoles from Tifton. This conclusion was supported not only from visually comparing the percentages of body mass lost during dehydration (fig. 3) but also from our analyses of

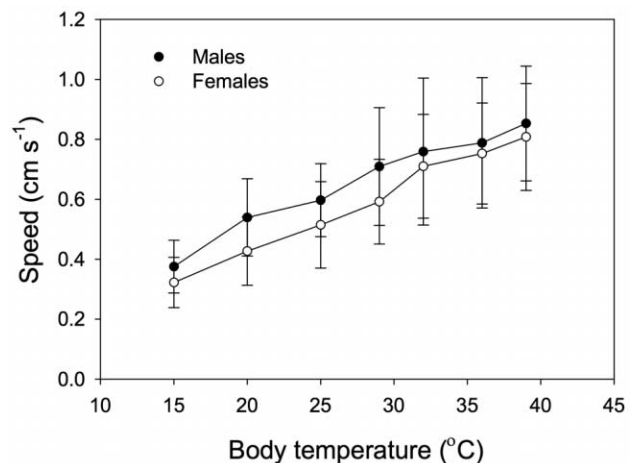


Figure 5. Speed increased with increasing temperature for brown anoles of both sexes. Circles and error bars denote means and standard deviations, respectively.

Table 3: Inferential statistics for the most likely general linear mixed model of endurance

Effect	Effect df	Error df	F	P
Intercept	1	44	7.12	.011
Snout-vent length	1	44	5.40	.025
Sex	1	44	3.05	.088
Population	2	44	2.12	.133
Snout-vent length × sex	1	44	3.87	.056
Snout-vent length × population	2	44	2.08	.138
Sex × population	2	44	.93	.403
Snout-vent length × sex × population	2	44	1.06	.354

Note. The standard deviation of endurance for anoles from Miami and Orlando was 1.4 and 2.0 times greater, respectively, than that of anoles from Tifton. This model fitted the data substantially better than the second-best model (ΔAIC [Akaike Information Criterion] = 10.4), which excluded the interaction among snout-vent length, sex, and population.

changes in body mass (table 1). Juveniles lost water at much higher rates compared to adults (fig. 3).

Metabolic Rate

The thermal sensitivity of metabolic rate varied among populations; this variation resulted from an indirect effect of body mass, since mean body mass differed among populations and a direct effect of population after controlling for body mass (table 2). For a given body mass, anoles from Tifton had the lowest rates of metabolism, whereas anoles from Orlando had the highest rates (fig. 4). Although populations differ in metabolic rate, there is no evidence for a latitudinal cline. The most likely model included an effect of sex/stage, as well as the effect of body mass; however, we cannot interpret this effect independently of the mass effect because the ranges of masses were widely separated among groups (note the clustering of data on the plots in fig. 4).

Sprint Speed

The relationship between temperature and sprint speed was nonlinear ($F_{6,348} = 107.40$, $P < 0.0001$; fig. 5), as one would expect from both theoretical and empirical perspectives (Huey and Stevenson 1979; Angilletta 2009). At temperatures between 15° and 29°C, warmer lizards generally ran faster. Above this thermal range, speed did not change appreciably with increasing temperature. Overall, males ran faster than females ($F_{1,58} = 5.78$, $P = 0.02$). The faster speed of males was probably not driven by their larger size, because snout-vent length was excluded from the most likely model of speed. Although anoles from different populations ran at similar speeds, a model that included the effect of population ($P = 0.28$) was similar to the most likely model ($\Delta\text{AIC} = 1.4$).

Endurance

Population was included in the most likely model of endurance, which indicated that the body size and sex influenced which population of lizards had the highest endurance (table 3). Nevertheless, these complex relationships explained little of the variation, suggesting that none of these factors was very important for endurance (fig. 6).

Acclimation of Critical Thermal Minima

For lizards from both Orlando and Miami, critical thermal minima acclimated during 18 wk of exposure to either low or high temperature (table 4). In both thermal treatments, critical thermal minima decreased over time, but the decrease was

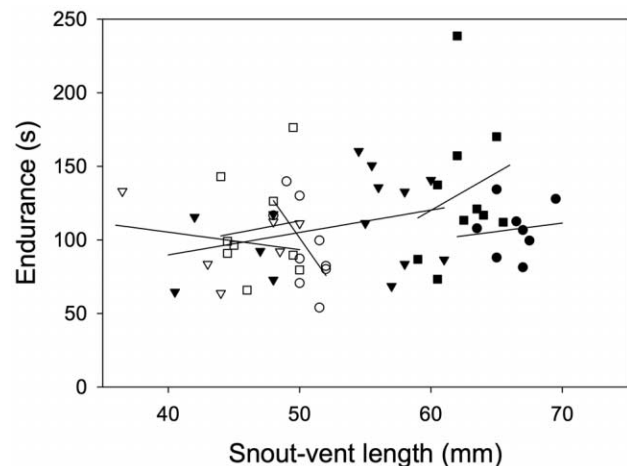


Figure 6. Larger brown anoles tended to have greater endurance; however, much of the variation was unexplained by size, population, or sex. Endurances of males and females are depicted by filled and open symbols, respectively. Brown anoles from Tifton, Orlando, and Miami are depicted by circles, squares, and triangles, respectively.

Table 4: Inferential statistics for the most likely general linear mixed model of critical thermal minima during acclimation to temperature

Effect	Effect df	Error df	F	P
Intercept	1	229	318.781	<.0001
Initial mass	1	229	3.285	.071
Population	1	229	.519	.472
Acclimation temperature	1	229	7.959	.005
Sex	1	229	.019	.891
Acclimation time	5	229	11.357	<.0001
Initial mass × population	1	229	12.821	.0004
Initial mass × acclimation temperature	1	229	3.833	.052
Initial mass × sex	1	229	1.884	.171
Initial mass × acclimation time	5	229	.767	.575
Population × acclimation temperature	1	229	6.525	.011
Population × sex	1	229	.034	.853
Population × acclimation time	5	229	.960	.443
Acclimation temperature × sex	1	229	2.558	.111
Acclimation temperature × acclimation time	5	229	16.407	<.0001
Sex × acclimation time	5	229	.866	.504
Initial mass × population × acclimation temperature	1	229	7.164	.008
Population × acclimation temperature × sex	1	229	2.944	.088
Initial mass × population × acclimation time	5	229	4.063	.002

greater in the low-temperature treatment. These different responses were evident within 2 wk and remained consistent throughout the duration of the experiment (fig. 7). Even after 18 wk at a low temperature, however, the mean critical thermal minima of lizards from Miami exceeded that of lizards from Orlando. By contrast, critical thermal minima for populations in the high-temperature treatment converged within 18 wk (fig. 7).

Acclimation of Water Loss

Rates of water loss acclimated during exposure to either low or high relative humidity (table 5). After 2 wk, anoles from Miami kept at low humidity dehydrated more slowly than did those kept at high humidity (fig. 8).

Discussion

We discovered differences in physiological phenotypes among invasive populations of the brown anole. Critical thermal minima and rates of metabolism and dehydration varied among populations (figs. 2–4), whereas critical thermal maxima, endurance, and sprint speeds did not. Latitudinal variation in critical thermal minima and rates of water loss matched those predicted from parallel clines in air temperature and relative humidity (fig. 1; Angilletta 2009). By contrast, the variation in metabolic rate among populations did not reflect our prediction based on the thermal cline. Critical thermal minima and rates of water loss both responded to short-term acclimation (figs. 7, 8), suggesting plasticity at least in part explains differences among populations for these traits. Below, we discuss how ad-

aptation, plasticity, and repeated colonization might have contributed to these patterns of physiological variation and whether these traits have helped this species to spread in the southeastern United States.

Lizards from Tifton, the northernmost population in our study, tolerated low temperatures better than lizards from other populations despite it being the most recently established population in our study (fig. 2). This ability can enhance the probability of survival during the colder winters in Tifton. Lethal temperatures are correlated with critical thermal minima in some taxa (Hori and Kimura 1998; Das et al. 2004), but we do not know whether this relationship also holds for brown anoles. The ecologically similar species *Anolis cristatellus* in Puerto Rico experienced reduced survival when held at lower-than-normal temperatures (i.e., 16°C for 19 d; Gorman and Hillman 1977). The native species of anole, *Anolis carolinensis*, shows similar latitudinal variation in low-temperature tolerance; lizards from Valdosta, Georgia, tolerate cold better than lizards from Orlando, Florida (Wilson and Echternacht 1987).

Native green and invasive brown anoles in Florida can lower their critical thermal minima after short-term exposure to low temperatures (Wilson and Echternacht 1987; Kolbe et al. 2012). In a previous study, acclimation decreased the mean critical thermal minimum by 2°C within 2 wk for anoles from Miami (Kolbe et al. 2012). Acclimation could account for phenotypic differences between recently introduced populations and their native-range sources, such as the distinct critical thermal minima of crested anoles (*Anolis cristatellus*) from Miami and Puerto Rico (Kolbe et al. 2012; Leal and Gunderson 2012). Our acclimation experiment revealed similar responses for lizards

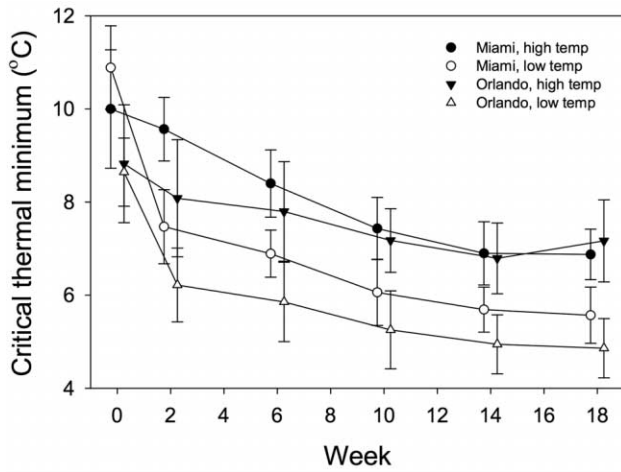


Figure 7. Brown anoles from Orlando and Miami tolerated lower temperatures after thermal acclimation. Critical thermal minima were measured repeatedly throughout 18 wk of acclimation to either high or low temperature (mean = 28.9° or 19.6°C, respectively). Means are offset to enhance visibility of error bars, which denote standard deviations.

from Miami and Orlando; however, lizards from Orlando still tolerated lower temperatures than did lizards from Miami after acclimation (fig. 8). This lack of convergence after 18 wk suggests a genetic difference between the populations. Still, additional experiments are needed to rule out the contribution of developmental plasticity during the embryonic and juvenile stages.

As with lower thermal tolerance, water loss rates conformed to predictions such that the northernmost population showed the greatest resistance to water loss (fig. 3). Tifton lizards lost water at about one-third of the rate of lizards from Miami, where relative humidity is less extreme (fig. 1). Short-term laboratory acclimation at low humidity reduced the rate of water loss for lizards from Miami (fig. 8), suggesting population-level differences may be due in part to plasticity. Anoles show variation in water loss rates both among populations (Hertz et al. 1979; Dmi’el et al. 1997; Gunderson et al. 2011) and among species (Sexton and Heatwole 1968; Hillman and Gorman 1977) in their native ranges. As in this study, most comparisons find greater resistance to water loss in populations from drier climates or microhabitats (Dmi’el et al. 1997; Gunderson et al. 2011).

Tropical ectotherms are thought to have limited ability to acclimate given their relatively stable environments (Janzen 1967). Indeed, populations of *A. cristatellus* from xeric and mesic areas in Puerto Rico did not alter their desiccation rates after exposures to different humidities (Gunderson et al. 2011). Moreover, lizards from the population in Puerto Rico that seeded an introduced population in Miami did not lower their thermal tolerance when exposed to low temperatures (Kolbe et al. 2012). By contrast, the temperate species, *A. carolinensis*, resists water loss better after exposure to low humidity (Ko-

bayashi et al. 1983; Kattan and Lillywhite 1989) and tolerates cold better after exposure to low temperatures (Wilson and Echternacht 1987; Kolbe et al. 2012). Although the tropical origin of the brown anole suggests that this species should be unable to acclimate its water loss rate or thermal tolerance, the environmental conditions in its invasive range may select for acclimation ability, as observed in *A. carolinensis*. Our laboratory experiments showed that both traits acclimate, suggesting that environmental conditions in the invasive range can quickly influence physiological phenotypes. Comparisons of acclimation capacities between invasive populations and source populations in Cuba would indicate whether acclimation capacity has evolved since the introduction or existed in the immigrants that founded the invasion (e.g., Kolbe et al. 2012).

Although metabolic rates differ among invasive populations of brown anoles, the pattern of differentiation was not predicted from latitudinal clines (fig. 4). Tifton consistently had the lowest metabolic rates across all temperatures, which contrasts with previous studies that showed lizards from more seasonal climates have higher metabolic rates (Tsuji 1988a, 1988b; Garland and Adolph 1991; Angilletta 2001b). Previous studies of three tropical species of anoles, including brown anoles from Miami (Rogowitz 1996a, 1996b), uncovered no altitudinal variation or thermal acclimation of metabolic rates. Furthermore, the temperate species (*A. carolinensis*) has only a limited ability to acclimate its resting metabolism to changing temperature (Gatten 1985; Gatten et al. 1988). Thus, differences in metabolic rate among invasive populations of brown anoles might not have resulted from plasticity. The lack of latitudinal variation in metabolism suggests the possibility of a nonadaptive explanation or at least a more complex adaptive explanation than we had envisioned. Morphological differences among invasive brown anole populations resulted from the pattern of admixture from multiple native-range sources (Kolbe et al. 2007b), which could potentially explain differences in metabolic rate. Although molecular genetic data exist to characterize the sources of our populations, no clear association between the invasion history and metabolic variation has emerged. A stronger test of the hypothesis that invasion history has driven variation in metabolic rate among populations requires sampling of more populations throughout the invasive range.

Biologists trying to connect physiological phenotypes to in-

Table 5: Inferential statistics for the most likely general linear mixed model of mass loss during acclimation to relative humidity

Effect	Effect df	Error df	F	P
Initial mass	1	198	8.70	.0036
Treatment	1	198	15.72	.0005
Trial	1	27	37.10	<.0001
Acclimation time	1	198	29.32	<.0001
Treatment × trial	1	198	8.56	.0038
Trial × acclimation time	1	198	6.01	.0151

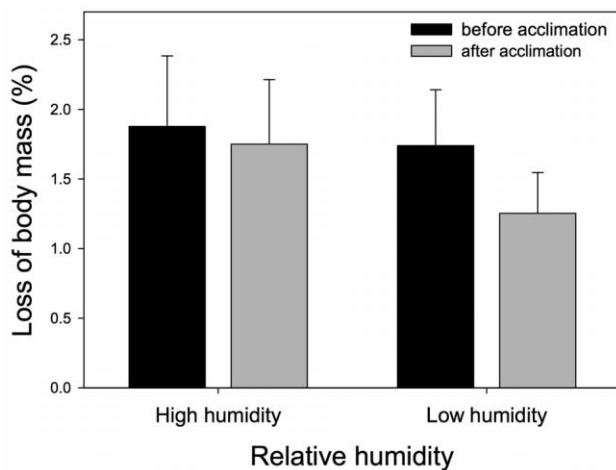


Figure 8. Brown anoles from Miami lost water at a slower rate after acclimation at low relative humidity. Error bars denote standard deviations.

vasion success have focused primarily on the properties of invading species alone or in comparison to those of native species (e.g., Chown et al. 2007; Schnell and Seebacher 2008). For example, Zerebecki and Sorte (2011) found invasive species of marine invertebrates tolerate a broader range of temperatures and higher maximal temperatures than do native species. These studies highlight that physiological traits may play a role in invasion success but do not address whether these traits have evolved within the invasive range. Yet, adaptation to novel environments and adaptive phenotypic plasticity can facilitate the spread of an invasive species. For example, cane toads at the southern edge of the invasive range of Australia enhanced their low-temperature tolerance through acclimation (Kolbe et al. 2010). By contrast, cane toads along the northern invasion path in Australia lacked variation in physiological traits associated with dispersal, including endurance, recovery from exercise, and properties of blood and muscle (Tracy et al. 2011), suggesting divergence in these traits was not required for range expansion in the north.

Similarly, some traits such as critical thermal maximum and sprint speed did not vary among brown anole populations in our study, suggesting suitable phenotypic values existed before expansion into new areas. These results emphasize the importance of assessing whether phenotypic variation among invasive populations is related to environmental gradients, such as temperature and precipitation. Such relationships could indicate that adaptation or adaptive phenotypic plasticity of physiological traits has played a role in range expansion during an invasion. The absence of distinct patterns can shift focus from physiology to behavior, which can also determine invasion success (Chapple et al. 2012). Ultimately, reciprocal transplant experiments are needed to determine whether the phenotypic variation, such as low-temperature tolerance, desiccation tolerance, and metabolic rate that we observed, actually confer a fitness advantage and extend the invasive range.

The variation in thermal physiology among invasive populations of *Anolis sagrei* contrasts with the evolutionary conservation of thermal physiology within other groups of lizards (Navas 2002; Youssef et al. 2008). For example, the thermal tolerance of *Sceloporus undulatus* does not vary according to the thermal conditions throughout its range (Buckley et al., unpublished manuscript). Similarly, poor relationships between climate and tolerance were observed among species of *Phymaturus* lizards (Cruz et al. 2009). Other researchers have argued that behavioral thermoregulation has minimized selective pressures in thermal physiology (Bogert 1949; Huey et al. 2003). Certainly, body temperatures during activity can remain remarkably stable within and among species that cover a wide climatic range (Andrews 1998; Buckley et al. in review). That said, other groups of lizards—such as Central and South American species of *Sceloporus* (Andrews 1998), *Liolaemus* (Rodríguez-Serrano et al. 2009), and *Tropidurus* (Kiefer et al. 2005; Labra et al. 2009)—maintain lower body temperatures in colder environments. Body temperatures in *Anolis* are often correlated with ambient temperatures suggesting that climatic gradients should translate into body temperature differences (e.g., Ruibal and Philibosian 1970; Clark and Kroll 1974; Huey and Webster 1976). Thus, *Anolis* seems to be among those groups that rely on physiological responses to environmental conditions, since both the native *A. carolinensis* (Wilson and Echternacht 1987) and the invasive *A. sagrei* (this study) exhibit latitudinal differences in low-temperature tolerance and dehydration resistance. Our study provides some evidence for physiological plasticity, but additional studies are needed to clarify how genetic and environmental factors contribute to these clines.

Efforts to model invasive species nonnative distributions require accurate estimates of physiological parameters (Kearney et al. 2008, 2009; Buckley et al. 2010). Population dynamics stem from physiological processes that affect growth, survival, and reproduction (Dunham et al. 1989). The rates of these physiological processes often vary among populations distributed along environmental clines (Beaupre 1993; Beaupre et al. 1993; Angilletta et al. 2001a, 2001b; Sears 2005). Genetic and environmental sources of physiological variation can either combine or counteract, leading to complex patterns of performance among populations (Conover and Schultz 1995). By accounting for this physiological variation, researchers have tailored projected impacts of climate change to specific phenotypes (Buckley 2008, 2010). In the same way, we can use information about physiological variation within species to better predict the outcomes of invasions.

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