

Tests of the contribution of acclimation to geographic variation in water loss rates of the West Indian lizard *Anolis cristatellus*

Alex R. Gunderson · Jeremy Siegel ·
Manuel Leal

Received: 9 January 2011 / Revised: 11 March 2011 / Accepted: 30 March 2011 / Published online: 23 April 2011
© Springer-Verlag 2011

Abstract Phenotypic plasticity can contribute to the process of adaptive radiation by facilitating population persistence in novel environments. West Indian *Anolis* lizards provide a classic example of an adaptive radiation, in which divergence has occurred along two primary ecological axes: structural microhabitat and climate. Adaptive plasticity in limb morphology is hypothesized to have facilitated divergence along the structural niche axis in *Anolis*, but very little work has explored plasticity in physiological traits. Here, we experimentally ask whether Puerto Rican *Anolis cristatellus* from mesic and xeric habitats differ in desiccation rates, and whether these lizards exhibit an acclimation response to changes in relative humidity. We first present microclimatic data collected at lizard perch sites that demonstrate that abiotic conditions experienced by lizards differ between mesic and xeric habitat types. In Experiment 1, we measured desiccation rates of lizards from both habitats maintained under identical laboratory conditions. This experiment demonstrated that desiccation rates differ between populations; xeric lizards lose water more slowly than mesic lizards. In Experiment 2, lizards from each habitat were either maintained under the conditions of Experiment 1, or under extremely low relative humidity. Desiccation rates did not differ between lizards from the same habitat maintained under different treatments and xeric lizards maintained lower desiccation rates than mesic lizards within each treatment. Our results demonstrate that *A. cristatellus* does

not exhibit an acclimation response to abrupt changes of hydric conditions, and suggest that tropical *Anolis* lizards might be unable to exhibit physiological plasticity in desiccation rates in response to varying climatic conditions.

Keywords Acclimation · Climate · Desiccation · Adaptive radiation · Geographic variation · *Anolis cristatellus* · Puerto Rico

Introduction

Phenotypes can vary in response to the environmental conditions individuals experience during their lifetime, a phenomenon known as phenotypic plasticity (reviewed in West-Eberhard 2003). The expression of phenotypic plasticity (or a lack thereof) can have important ecological and evolutionary implications for populations, including influences on the interactions between species (Agrawal 2001), the evolution of phenotypic traits (Ghalambour et al. 2007), and the probability that species persist in the face of anthropogenic climate change (Stillman 2003). In some cases, plasticity may be mal-adaptive or neutral (DeWitt et al. 1998; Ghalambour et al. 2007). In other cases, plasticity appears to be adaptive in that the phenotype expressed in a particular environment is predicted to increase fitness relative to the phenotypes expressed in other environments. For example, the maximum critical lethal temperature of many fish increases after exposure to warm temperatures, a change that should reduce the probability of mortality under natural conditions (Reynolds and Casterlin 1979).

Phenotypic plasticity could play an important role in the process of adaptive radiation by facilitating the persistence of populations that encounter new environmental challenges (West-Eberhard 2003; Sol et al. 2005; Pfennig and

Communicated by G. Heldmaier.

A. R. Gunderson (✉) · J. Siegel · M. Leal
Biology Department, Duke University, Durham,
NC 27708, USA
e-mail: alexander.gunderson@duke.edu

Pfennig 2009), a scenario that has been hypothesized for lizards in the genus *Anolis* (Losos et al. 2000). *Anolis* lizards have adaptively radiated on the Greater Antillean islands along two major ecological axes: structural niche and climatic niche (Williams 1972, 1983). Adaptive divergence in a suite of morphological traits, including limb size, is correlated with the occupation of different structural niches (e.g., tree trunks, twigs, grasses) (Losos 2009). Experimental manipulations with *A. carolinensis* and *A. sagrei* have demonstrated that limb size can change in response to the structural habitat (i.e., the distribution of available perch diameters) under which juveniles develop (Losos et al. 2000; Kolbe and Losos 2005). This developmental plasticity appears adaptive as it results in limbs predicted to be beneficial on the perch types used by influencing speed and stability (Losos et al. 2000, 2009; Spezzano and Jayne 2004).

The other main axis of *Anolis* divergence is the climatic niche axis (Williams 1972; Losos 2009). The islands of the Greater Antilles are topographically complex and exhibit extensive variation in thermal conditions and rainfall, including high elevation rain forests and low elevation desert scrublands. Most species of anole occupy a distinct climatic niche within each island (Ruibal 1961; Rand 1964; Williams 1972; Huey and Webster 1976; Hertz 1992a, b), and physiological differences exist between species inhabiting different niches. For example, species found in warm, dry regions can tolerate higher temperatures and have lower rates of water loss than species from cool moist regions (Sexton and Heatwole 1968; Huey and Webster 1976; Hillman and Gorman 1977; Hertz 1979, 1980; Hillman et al. 1979; Dmi'el et al. 1997; Perry et al. 1999, 2000). Historically, these differences had been interpreted as having a genetic basis. However, the degree of plasticity that can be exhibited by physiological traits has been little explored in anoles. For example, laboratory tests of acclimation capacity of cutaneous water loss rate, a trait thought to be important for maintaining water balance, have been conducted under controlled climatic conditions on only one species of anole, *A. carolinensis* (Kobayashi et al. 1983; Kattan and Lillywhite 1989). In this species, individuals significantly reduce their rates of cutaneous water loss after only 1 week of exposure to low-humidity conditions (Kobayashi et al. 1983; Kattan and Lillywhite 1989). However, whether or not the acclimation response observed in *A. carolinensis*, which is the only member of the West Indian radiation with an exclusively temperate distribution (Losos 2009), occurs in other anoles remains an open question.

Here, we evaluate geographic variation and phenotypic plasticity in water loss rates in the Puerto Rican *A. cristatellus*. With respect to phenotypic plasticity, we explicitly test the ability of adult lizards to acclimate to changes in relative humidity. Previous research on water loss rates of

A. cristatellus on the British Virgin Islands has demonstrated that populations from arid regions tend to have lower rates of water loss than those from wetter regions (Dmi'el et al. 1997; Perry et al. 1999, 2000). The authors suggested that this variation was at least partially due to phenotypic plasticity, although in a common garden experiment using outdoor enclosures they found no change in water loss rates of lizards from different regions over a 1-month period (Perry et al. 2000). Thus, it is unclear if water loss rates of *A. cristatellus* can exhibit an acclimation response to changing climatic conditions. Furthermore, rigorous tests for acclimation of water loss rates under controlled climatic conditions are currently lacking for a tropical anole.

In this study, we conducted experiments under controlled laboratory conditions to address the following questions. First, do populations of *A. cristatellus* inhabiting distinct climatic conditions on the island of Puerto Rico differ in water loss rates? Second, can individuals of *A. cristatellus* adjust their water loss rates in response to changes in humidity regimes? To achieve these goals we measured water loss rates on two populations of *A. cristatellus* inhabiting distinct habitat types (Rand 1964; Heatwole 1976): warm xeric forest with low rainfall and cooler mesic forest with relatively high rainfall (Ewel and Whitmore 1973; Daly et al. 2003). Since water loss rates of individuals in the field are dictated by the conditions of their immediate surrounding, and not by average climatic conditions across a large geographic range (Hertz 1980), we first present data on the environmental conditions (air temperature, wind speed, relative humidity) experienced by lizards at their perch sites in the field. Next, we present data from experiments testing whether the water loss rates of individuals are correlated with habitat type. We predict that individuals from a xeric habitat should lose water at a lower rate than individuals from a mesic habitat. Finally, we evaluate if lizards are able to acclimate to changes in relative humidity. To do so we conducted a controlled experimental manipulation in which individuals were exposed to two distinct humidity regimes, while keeping the temperature constant across treatments. In particular, we ask whether or not mesic lizards converge on water loss rates of xeric lizards when individuals from both habitat types are housed under low-humidity conditions.

Methods

Study species and locations

Anolis cristatellus is a small-to-medium sized (maximum snout-vent length (SVL) for males 76 mm, females 56 mm) arboreal lizard typically found on the ground or on

tree trunks up to 2 m in height (a member of the “trunk-ground” ecomorph category, Williams 1983). *A. cristatellus* is endemic to the islands of the greater Puerto Rican Bank. On the island of Puerto Rico *A. cristatellus* can be found from sea level to mid-elevation (~1,000 m) inhabiting mesic and xeric habitats (Rand 1964; Heatwole 1976).

We collected adult males and females of *A. cristatellus* and sampled habitat conditions at Cambalache State Forest (18°26'35"N; 66°35'47"W), a mesic forest located in the karst region in the northwestern part of the island, and at Guánica State Forest (17°58'19"N; 66°52'06"W), a xeric forest on the southern coast. Cambalache State Forest can be characterized as a woodland with a mostly closed canopy and mean annual rainfall of 1,500 mm. Guánica State Forest is a xeric desert scrubland that receives only 760 mm of annual rainfall (Helmer et al. 2002). In addition, operative temperatures and lizard body temperatures are more than 3°C higher in Guánica than Cambalache during both the wet and dry season (A.R. Gunderson and M. Leal, unpubl. data).

Perch microclimates

We measured air temperature, wind speed, and relative humidity at perch sites occupied by lizards from 14 to 30, July 2010. Data were collected at the perch sites of 176 individuals at the mesic site and 173 individuals at the xeric site. In each forest we slowly walked through the habitat from 0745 to 1715 hours, a period when lizards are active, looking for lizards. When a lizard was found, we collected data on the sex, perch height, lizard orientation, basking status, and weather conditions before approaching the lizard. At the perch site, air speed, air temperature, and relative humidity were measured with an anemometer/hygrometer (Omega, Inc., model HHF11) that was carried with its sensor exposed to decrease equilibration time. Air speed was taken over a period of approximately 10 s with the probe held perpendicular to the wind direction approximately 10 cm from the perch. The probe was then allowed to equilibrate for approximately 2 min, at which time air temperature and relative humidity were recorded.

Experiment 1

We collected adult individuals at each site from August 22 to 23, 2009, and brought them back to our laboratory at Duke University. Lizards were housed individually in 29 × 21 × 21 cm (L × W × H) plastic cages with a wooden dowel provided as a perch. Cages were kept in a climate-controlled growth chamber (Environmental Growth Chambers, model M25-3400) set at 28°C with a relative humidity (RH) of 60% (water vapor deficit, 1.50 kPa) and a 12L:12D light cycle with full spectrum light provided

during daylight hours. All cages had mesh tops to facilitate the equilibration of cage air temperature and relative humidity with the growth chamber. Lizards were fed crickets supplemented with calcium powder three times weekly and watered twice daily during weekdays and once per day on weekends.

After approximately 3 months in captivity, we measured water loss rates by quantifying the change in body mass of lizards placed in an incubator (Percival, Inc., model I30NLC8) set at a constant temperature and humidity (following similar methodologies in Hertz 1979, 1980; Hillman and Gorman 1977; Kobayashi et al. 1983; Kattan and Lillywhite 1989; Perry et al. 2000). Data were collected from 26 individuals (15 males and 11 females) in the mesic habitat and from 24 individuals (15 males and 9 females) in the xeric habitat. Before placing the lizards inside the incubator, individuals were housed individually in modified 414 ml Tupperware containers with mesh tops and bottoms to allow for airflow. The day before the experiment, all containers were placed inside the incubator to dry them. On the day of the experiment, the mass of each container was measured immediately before and after a lizard was placed in it to calculate initial lizard mass. Immediately after taking these measurements, we placed containers with lizards on racks inside the incubator. The incubator was set at 30°C with a RH of 10% (RH ranged from 8 to 11% during the experiments). The 30°C temperature is within the preferred-temperature range of *A. cristatellus* from both mesic and xeric habitats (Hertz et al. 1993), and falls within the range of temperatures experienced by *A. cristatellus* at each site (A.R. Gunderson and M. Leal, unpubl. data). The interior of the incubator was kept in total darkness, which, combined with the relatively small volume of the containers, should have drastically reduced if not eliminated lizard activity during the experiment. We weighed the containers with lizards every 2 h over an 8 h period. Mass was taken with an electric balance sensitive to 0.0001 g (Ohaus Inc., Adventurer Pro). If a lizard defecated during a particular 2-h period, we excluded the mass lost from that period in our calculations of water loss.

We calculated a number of metrics of water loss commonly used to compare differences in water loss rates among populations and species: mass-specific water loss (mg/g/h), percent body weight lost (BW%/h), area-specific water loss (mg/cm²/h), and cutaneous resistance (*R*, s/cm). To calculate area-specific water loss, we calculated the surface area of each lizard using the following equation: $A = 16.59BW^{0.53}$ where BW is body weight in grams. This conversion was empirically derived specifically for *A. cristatellus* (Dmi'el et al. 1997). We assumed that 75% of the water loss we recorded occurred across the integument, as this has also been empirically determined for *A. cristatellus* at 30°C (Dmi'el et al. 1997). Similar values

of percent cutaneous water loss have been shown for other lizards (Bentley and Schmidt-Nielsen 1966; Eynan and Dmi'el 1993). Skin resistance to water loss was calculated following Lillywhite and Sanmartino (1993).

Experiment 2

We conducted a second experiment to determine whether exposure to extremely low humidity would induce acclimation of desiccation resistance. We randomly divided the lizards used in the first experiment into two groups. One group continued to be maintained under the standard laboratory conditions described above (60% RH, 30°C, 12L:12D), whereas the other group was placed in a second climate-controlled room, in which all of the abiotic conditions were the same as in the other group except that RH was maintained at 30% (water vapor deficit, 2.63 kPa). Feeding and watering continued in both groups as described in Experiment 1. Lizards were maintained under these conditions for 2 weeks, at which time water loss rates of all individuals were estimated using the methodology described in Experiment 1. The congeneric *A. carolinensis* shows an acclimation response after experiencing low-humidity conditions for only 1 week (Kobayashi et al. 1983; Kattan and Lillywhite 1989).

Statistical analyses

We tested our hypotheses using data on area-specific rates of water loss. We also present other metrics of water loss for comparison with other studies (Table 2). Mass-specific water loss correlates negatively with body size, most likely due to the differential scaling of mass and surface area (discussed in Hertz 1980). Xeric lizards were larger than mesic lizards (Table 2), and, thus, mass-specific metrics are biased towards detecting a difference between lizards from the two habitats based on size alone.

The data were not normally distributed and none of the transformations we used produced a normally distributed data set. Therefore, after confirming homogeneity of variances among groups (F tests, all $P > 0.050$), we used non-parametric Wilcoxon rank-sum tests on untransformed data for all comparisons. All statistical analyses were done using the R statistical programming package version 2.9.2 (R Development Core Team 2009).

Results

Perch site microclimates

The microclimate conditions present at the lizards perch sites differ between habitat types. On average, lizards in the xeric

Table 1 Summary statistics of environmental data (mean \pm SE) collected at the perch sites of lizards in each habitat type. Data are partitioned by sex and weather conditions at the time of sampling

	N	T_{air}	Wind speed	RH
Mesic habitat				
Female/cloudy	7	26.4 (0.1)	0.1 (0.1)	84.9 (0.9)
Female/sunny	115	28.0 (0.1)	0.6 (0.0)	83.7 (0.6)
Male/cloudy	5	26.7 (0.1)	0.0 (0.0)	86.6 (0.9)
Male/sunny	49	28.3 (0.2)	0.7 (0.1)	81.2 (0.9)
Total	176	28.0 (0.1)	0.6 (0.0)	83.1 (0.5)
Xeric habitat				
Female/cloudy	41	29.3 (0.2)	0.6 (0.1)	72.9 (1.0)
Female/sunny	71	30.8 (0.2)	0.8 (0.0)	69.1 (0.8)
Male/cloudy	28	29.7 (0.3)	0.7 (0.1)	73.3 (1.1)
Male/sunny	33	30.4 (0.4)	0.9 (0.1)	70.1 (1.5)
Total	173	30.2 (0.1)	0.8 (0.0)	70.9 (0.5)

N sample size, T_{air} air temperature (°C), wind speed (m/s), RH relative humidity

habitat experienced air temperatures 2.2°C warmer than lizards in the mesic habitat ($P < 0.001$) (Table 1). In addition, relative humidity at xeric perch sites averaged 12.2% lower than at mesic perch sites ($P < 0.001$) (Table 1). Similarly, wind speed was significantly higher in the xeric habitat, by an average of 0.2 m/s ($P = 0.001$) (Table 1).

Experiment 1

Among individuals from the same habitat type there were no significant differences in area-specific water loss rates between males and females (xeric lizards, $P = 0.2895$; mesic lizards, $P = 0.2171$) (Table 2). Thus, data from males and females are combined for all subsequent analyses. Lizards from the xeric habitat lost water at a significantly lower rate than individuals from the mesic habitat ($P = 0.003$) (Table 2), congruent with the general trend observed in anoles in which individuals from xeric habitats are more resistant to desiccation.

Experiment 2

Area-specific water loss rates of lizards from the same habitat type did not differ between individuals placed at 30% RH and those at 60% RH (mesic lizards, $P = 0.851$; xeric lizards, $P = 0.426$) (Table 3), demonstrating the lack of an acclimation response to changes in hydric conditions over a 2-week period. In accordance with the results from Experiment 1, water loss rates of xeric lizards were lower than those of mesic lizards in both treatments (30% RH, $P = 0.058$; 60% RH, $P = 0.008$) (Table 3). Population estimates of water loss rates increased between Experiment

Table 2 Summary statistics (mean ± SE) for water loss rates of mesic and xeric individuals of *A. cristatellus* maintained under identical conditions of temperature and humidity (“Experiment 1”)

	<i>N</i>	Lizard mass (g)	MSWL	BW%/h	ASWL	<i>R</i>	Predicted survival time (h)
Mesic							
Males	15	5.7 (0.1)	1.66 (0.08)	0.13 (0.01)	0.18 (0.01)	579 (27)	199
Females	11	1.9 (0.1)	2.62 (0.18)	0.20 (0.02)	0.16 (0.01)	651 (45)	125
Combined	26	–	–	–	0.17 (0.01)	609 (25)	–
Xeric							
Males	15	6.4 (0.2)	1.31 (0.07)	0.17 (0.01)	0.14 (0.01)	734 (44)	251
Females	9	2.2 (0.1)	2.00 (0.07)	0.26 (0.02)	0.13 (0.01)	805 (69)	147
Combined	24	–	–	–	0.14 (0.01)	760 (37)	–

MSWL mass-specific water loss rate (mg/g/h), BW%/h rate of water loss expressed as a percentage of body weight (BW) per hour, ASWL area-specific water loss rate (mg/cm²/h), *R* resistance (skin + boundary layer) of lizards to water loss (s/cm), Predicted survival time the estimated time, in hours, that an average individual would survive before reaching a lethal dehydration state under experimental conditions

Table 3 Summary statistics (mean ± SE) for water loss rates of mesic and xeric individuals of *A. cristatellus* maintained under different drying regimes (“Experiment 2”)

Treatment	<i>N</i>	ASWL	<i>R</i>
Mesic			
60% RH	10	0.28 (0.02)	367 (24)
30% RH	14	0.27 (0.02)	385 (28)
Xeric			
60% RH	10	0.20 (0.02)	530 (50)
30% RH	11	0.22 (0.02)	467 (28)

ASWL area-specific rates of water loss (mg/cm²/h), *R* resistance (skin + boundary layer) of lizards to water loss (s/cm)

1 and Experiment 2, irrespective of treatment or habitat of origin (Tables 2, 3).

Discussion

Our findings are consistent with the general consensus that species or populations living under distinct desiccation regimes exhibit different physiological capacities to resist water loss (Lillywhite 2006). The microhabitat data demonstrate that the perch sites occupied by mesic and xeric lizards differ significantly in abiotic conditions that influence desiccation rates. Air temperatures and wind speed were highest and relative humidity was lowest at xeric perch sites (Table 1). In addition, xeric lizards have higher body temperatures than mesic lizards (Hertz 1992a, A.R. Gunderson and M. Leal, unpubl. data). Theoretically, this confluence of factors should cause xeric lizards to experience a stronger evaporative force driving water from their bodies than mesic lizards (Hertz 1980; Eynan and Dmi’el 1993).

As predicted, given the microclimate measurements and the general abiotic characteristics of each habitat type, we

found that individuals of *A. cristatellus* from the dry xeric habitat lost water more slowly than lizards from the moist mesic habitat (Table 2). This difference was present even though lizards from both populations were maintained under identical conditions (i.e., 28°C, 60% RH) for over 3 months before the experiment. By keeping the lizards under the same conditions for such a long period, differences in water loss rates that resulted from ecological conditions experienced before being captured should be minimized, if not completely removed. Moreover, the difference between mesic and xeric lizards was maintained irrespective of the conditions under which the lizards were maintained over a 2-week period (Table 3). This finding supports a lack of a physiological acclimatory response to changes in desiccation regimes. It has also been shown that metabolic rate does not acclimate to temperature in *A. cristatellus* (Rogowitz 1996). Taken together these findings suggest that individuals of *A. cristatellus* from the island of Puerto Rico are unable to exhibit a rapid physiological acclimation response to changing climatic conditions.

We are somewhat at a loss to explain the overall increase in water loss rates between Experiment 1 and Experiment 2. It is possible that the physiological ability to resist water loss decreases with time in captivity. Current empirical evidence addressing this point is lacking in anoles and lizards in general; however, the mass of lizards did not change significantly from Experiment 1 to Experiment 2 (data not shown), so it is unlikely that overall physiological condition decreased over time. Regardless of the overall difference in water loss rates between Experiment 1 and Experiment 2, xeric lizards lost water more slowly than mesic lizards when maintained under identical conditions in both experiments and water loss rates did not differ between individuals from the same habitat type placed under different conditions in Experiment 2. Thus, our data clearly suggest that the populations differ in their ability to

resist water loss and that *A. cristatellus* cannot physiologically acclimate to different hydric regimes.

Our results are consistent with previous research on West Indian anoles in suggesting that acclimation is not sufficient to account for patterns of water loss rate variation found between species or populations. In the few species studied thus far, negative correlations between habitat aridity and water loss rates have been found even if lizards from each population are exposed to identical climatic conditions preceding water loss measurement (Hertz 1979, 1980; Hillman et al. 1979; Perry et al. 2000). A series of previous studies has suggested that geographic variation in water loss rates of *A. cristatellus* from the British Virgin Islands is at least partially explained by acclimatory responses to climatic variation (Dmi'el et al. 1997; Perry et al. 1999, 2000); however, no acclimation was detected when individuals from different regions were placed in a common garden (an enclosure placed in the field) (Perry et al. 2000). A field common garden experiment has been used to address acclimation in only one other tropical *Anolis*, the Lesser Antillean *A. roquet* (Hillman et al. 1979). In this study, water loss rates of lizards from xeric, intermediate, and mesic habitats were shown to decrease after being placed in a common garden (a greenhouse in the field). However, in this study the acclimation conditions were unknown because the climatic conditions in the enclosure were not controlled nor measured systematically (Hillman et al. 1979). Thus, whether or not the changes observed were in the direction predicted by the acclimatory conditions is unknown. In general, our laboratory data and those of Perry et al. (2000) suggest that West Indian *Anolis* are unable to acclimate to changes in hydric conditions. In this respect tropical anoles appear to differ from the temperate *A. carolinensis*, which shows a reduction in cutaneous water loss rates after only 1 week of exposure to low-humidity conditions (Kobayashi et al. 1983; Kattan and Lillywhite 1989). Further research needs to evaluate if conditions experienced during embryonic or juvenile stages can contribute to intraspecific differences in physiological traits in anoles (Warner and Andrews 2002).

Our results suggest two non-mutually exclusive mechanisms by which organisms from arid environments can limit rates of water loss. The first is to simply reduce the rate at which water evaporates across the skin (Tables 2, 3). This is generally achieved by increasing the lipid content of the integument (Lillywhite 2006), although variation in the size and morphology of scales might also contribute to differences in water loss (Malhotra and Thorpe 1997; Thorpe et al. 2005; Calsbeek et al. 2006). However, in anoles there is no clear pattern with regard to scale size or number and habitat climatic conditions (reviewed in Malhotra and Thorpe 1997; Calsbeek

et al. 2006). In addition, a study evaluating geographic variation in *A. cristatellus* scalation found no differences in scale size or total scale number between the populations we sampled (Heatwole 1976). The second is to attain a larger size. Desiccation tolerance is often measured as the vital limit, or the percent of original body mass at which an organism dies from dehydration (Hertz et al. 1979; Hertz 1980). Larger individuals have a lower surface-area to volume ratio, so all else being equal, a large individual will approach its vital limit more slowly than a small individual will (Hertz 1980). In our sample, *A. cristatellus* from the xeric habitat were larger than those from the mesic habitat when comparing within sexes (*t* test, $P < 0.001$ for both males and females) (this pattern also holds when utilizing a larger data set, M. Leal and A.R. Gunderson, unpubl. data).

We used data from Experiment 1 to predict how long an “average” male and female individual from each habitat would be able to survive under the experimental conditions used in our study, using a previously published vital limit of 67% of body weight for *A. cristatellus* (Hertz 1979). An average xeric male would survive over 2 days longer than an average mesic male, whereas an average xeric female would survive almost one full day longer than an average mesic female (Table 2). Due to their small size, females are at a significant survival time disadvantage when compared to males. In fact, a mesic male is predicted to survive 2 days longer than a xeric female. This raises the question of how females, not to mention hatchlings of either sex, deal with this inherent disadvantage of small size. One possibility is that females and smaller individuals seek out areas that are more humid and/or cooler than large males. However, our microclimate measurements showed that neither air temperature nor relative humidity differed between the perch sites used by males and females (all $P > 0.05$, Table 1). In addition, there is no significant difference in body temperatures of male and female *A. cristatellus* (A.R. Gunderson and M. Leal, unpubl. data). Females may also have a lower vital limit or excrete smaller relative amounts of water. How smaller lizards deal with water loss rates in anoles is an open question that deserves further investigation.

Our results demonstrate that adult individuals of *A. cristatellus* are unable to show an acclimatory response to changes in humidity regimes, suggesting that phenotypic plasticity is not the main mechanism leading to differences in water loss rates between individuals inhabiting distinct ecological conditions. Thus, differences in desiccation resistance among populations of *A. cristatellus* may have a genetic basis. Further work is required to understand the interactions between plasticity and heritable variation in physiological traits in the adaptive radiation of anoles, in which interspecific differences in physiology are well

accepted as a major contributor to species diversity by allowing species to differentiate along a climatic niche axis (Losos 2009).

Acknowledgments We would like to thank Ray Huey, Paul Hertz, Brian Powell, David Steinberg, and three anonymous reviewers for providing helpful comments that improved the manuscript. We would also like to thank the Mata de Plátano (Intermerican University, Bayamón) and Isla Magueyes (University of Puerto Rico, Mayaguez) field stations, and Tony and Joan at T.J. Ranch for logistical support while in Puerto Rico. We are very grateful to the Departamento de Recursos Naturales y Ambientales of Puerto Rico, which provided all the necessary permits for conducting fieldwork. We followed the Recommendations for the Care of Amphibians and Reptiles (Pough 1991) in the treatment of all animals used in this study. The research presented here was approved by the Institutional Animal Care of Duke University. This work was supported by a Mellon Dissertation Research Grant from the Center for Latin American and Caribbean Studies at Duke University and a Bryden Graduate Student Research Grant from the North Carolina Academy of Sciences to ARG.

References

- Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. *Science* 294:321–326
- Bentley PJ, Schmidt-Nielsen K (1966) Cutaneous water loss in reptiles. *Science* 151:1547–1549
- Calsbeek R, Knouft JH, Smith TB (2006) Variation in scale numbers is consistent with ecologically based natural selection acting within and between lizard species. *Evol Ecol* 20:377–394
- Daly C, Helmer EH, Quiñones M (2003) Mapping the climate of Puerto Rico, Vieques and Culebra. *Int J Climatol* 23:1359–1381
- DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends Ecol Evol* 13:77–81
- Dmi'el R, Perry G, Lazell J (1997) Evaporative water loss in nine insular populations of the lizard *Anolis cristatellus* group in the British Virgin Islands. *Biotropica* 29:111–116
- Ewel JJ, Whitmore JL (1973) The ecological life zones of Puerto Rico and the U.S. Virgin Islands. Forest Service Research Paper ITF-18
- Eynan M, Dmi'el R (1993) Skin resistance to water loss in agamid lizards. *Oecologia* 95:290–294
- Ghalambour CK, McKay JM, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 21:394–407
- Heatwole H (1976) Herpetogeography of Puerto Rico VII. Geographic variation in the *Anolis cristatellus* complex in Puerto Rico and the Virgin Islands. *Occas Pap Mus Nat Hist Univ Kansas* 46:1–18
- Helmer EH, Ramos O, López TDM, Quiñones M, Diaz W (2002) Mapping the forest type and land cover of Puerto Rico, a component of the Caribbean biodiversity hotspot. *Carib J Sci* 38:165–183
- Hertz PE (1979) Sensitivity to high temperatures in three West Indian grass anoles (Sauria, Iguanidae), with a review of heat sensitivity in the genus *Anolis*. *Comp Biochem Physiol* 63:217–222
- Hertz PE (1980) Responses to dehydration in *Anolis* lizards sampled along altitudinal transects. *Copeia* 1980:440–446
- Hertz PE (1992a) Temperature regulation in Puerto Rican *Anolis* lizards: a field test using null hypotheses. *Ecology* 73:1405–1417
- Hertz PE (1992b) Evaluating thermal resource partitioning by sympatric lizards *Anolis cooki* and *A cristatellus*: a field test using null hypotheses. *Oecologia* 90:127–136
- Hertz PE, Arce-Hernandez A, Ramirez-Vasquez J, Tirado-Rivera W, Vazquez-Vives L (1979) Geographical variation of heat sensitivity and water loss rates in the tropical lizard, *Anolis gundlachi*. *Comp Biochem Physiol* 62:947–953
- Hertz PE, Huey RB, Stevenson RD (1993) Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am Nat* 142:796–818
- Hillman SS, Gorman GC (1977) Water loss, desiccation tolerance, and survival under desiccating conditions in 11 species of Caribbean *Anolis* evolutionary and ecological implications. *Oecologia* 29:105–116
- Hillman S, Gorman GC, Thomas R (1979) Water loss in *Anolis* lizards: evidence for acclimation and intraspecific differences along a habitat gradient. *Comp Biochem Physiol* 62:491–494
- Huey RB, Webster TP (1976) Thermal biology of *Anolis* lizards in a complex fauna: the *cristatellus* group on Puerto Rico. *Ecology* 57:985–994
- Kattan GH, Lillywhite HB (1989) Humidity acclimation and skin permeability in the lizard *Anolis carolinensis*. *Physiol Zool* 62:593–606
- Kobayashi D, Mautz WJ, Nagy KA (1983) Evaporative water loss: humidity acclimation in *Anolis carolinensis* lizards. *Copeia* 1983:701–704
- Kolbe JJ, Losos JB (2005) Hind-limb length plasticity in *Anolis carolinensis*. *J Herpetol* 39:674–678
- Lillywhite HB (2006) Water relations of tetrapod integument. *J Exp Biol* 209:202–226
- Lillywhite HB, Sanmartino V (1993) Permeability and water relations of hygroscopic skin of the file snake, *Acrochordus granulatus*. *Copeia* 1993:99–103
- Losos JB (2009) Lizards in an evolutionary tree: ecology and adaptive radiation of anoles. University of California Press, Berkeley
- Losos JB, Creer DA, Glossip D, Goellner R, Hampton A, Roberts G, Haskell N, Taylor P, Ettling J (2000) Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution* 54:301–305
- Malhotra A, Thorpe RS (1997) Microgeographic variation in scalation of *Anolis oculatus* (Dominica, West Indies): a multivariate analysis. *Herpetologica* 53:49–62
- Perry G, Dmi'el R, Lazell J (1999) Evaporative water loss in insular populations of the *Anolis cristatellus* group (Reptilia: Sauria) in the British Virgin Islands. II. The effects of drought. *Biotropica* 31:337–343
- Perry G, Dmi'el R, Lazell J (2000) Evaporative water loss in insular populations of *Anolis cristatellus* (Reptilia: Sauria) in the British Virgin Islands. III. Response to the end of drought and a common garden experiment. *Biotropica* 32:722–728
- Pfennig KS, Pfennig DW (2009) Character displacement: ecological and reproductive responses to a common evolutionary problem. *Q Rev Biol* 84:253–276
- Pough FH (1991) Recommendations for the care of amphibians and reptiles in academic institutions, vol 33. National Academic Press, pp S1–S21
- R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Austria
- Rand AS (1964) Ecological distribution in anoline lizards of Puerto Rico. *Ecology* 45:745–752
- Reynolds WW, Casterlin ME (1979) Behavioral thermoregulation and the “Final Referendum” paradigm. *Am Zool* 19:211–224
- Rogowitz GL (1996) Evaluation of thermal acclimation of metabolism in two eurythermal lizards, *Anolis cristatellus* and *A. sagrei*. *J Therm Biol* 21:11–14
- Ruibal R (1961) Thermal relations of five species of tropical lizards. *Evolution* 15:98–111

- Sexton OJ, Heatwole H (1968) An experimental investigation of habitat selection and water loss in some anoline lizards. *Ecology* 49:762–767
- Sol D, Stirling DG, Levebvre L (2005) Behavioral drive or behavioral inhibition in evolution: subspecific diversification in Holarctic passerines. *Evolution* 59:2669–2677
- Spezzano LC, Jayne BC (2004) The effects of surface diameter and incline on the hindlimb kinematics of an arboreal lizard (*Anolis sagrei*). *J Exp Biol* 207:2115–2131
- Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. *Science* 301:65
- Thorpe RS, Reardon JT, Malhotra A (2005) Common garden and natural selection experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*). *Am Nat* 165:495–504
- Warner DA, Andrews RM (2002) Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biol J Linn Soc* 76:105–124
- West-Eberhard MJ (2003) *Developmental plasticity and evolution*. Oxford University Press, Oxford
- Williams EE (1972) The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. *Evol Biol* 6:47–89
- Williams EE (1983) Ecomorphs, faunas, island size, and diverse end points in island radiations of *Anolis*. In: Huey RB, Pianka ER, Schoener TW (eds) *Lizard ecology: studies of a model organism*. Harvard University Press, Cambridge, pp 326–370