



Geographical variation in morphology and its environmental correlates in a widespread North American lizard, *Anolis carolinensis* (Squamata: Dactyloidae)

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The green anole, *Anolis carolinensis*, has long been an important model organism for studies of physiology and behaviour, and recently became the first reptile to have its genome sequenced. With a large and environmentally heterogeneous distribution, especially in relation to well-studied Antillean relatives, *A. carolinensis* is also emerging as an important organism for novel studies of geographical differentiation and adaptation. In the present study, we quantify the degree of morphological variation in this species and test for environmental correlates of this variation. We also examine adherence to Bergmann's and Allen's rule, two eco-geographical principles that have been well studied over large species ranges. We sampled from 14 populations across the distribution of the species in North America and measured 28 distinct morphological traits. We also collected a suite of environmental variables for each site, including those related to temperature, precipitation, and vegetation. Ultimately, we found a large degree of geographical variation in morphology, with head traits contributing the most to differences among populations. Morphological variation was correlated with variation in temperature, precipitation, and latitude across sites. We found no support for reverse Bergmann's rule typical of squamates, although we did find a trend of reverse Allen's rule. Ultimately, the present study provides a novel look at *A. carolinensis* and establishes it as a strong candidate for further studies of variation and adaptation over a large range. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 117, 760–774.

ADDITIONAL KEYWORDS: Allen's rule – Bergmann's rule – green anole.

INTRODUCTION

The green anole, *Anolis carolinensis* (Voigt 1832), is one of most common reptiles in the south-eastern USA and has a large distribution, ranging from Florida to Texas and reaching north to Oklahoma, Tennessee, and North Carolina (Campbell-Staton *et al.*, 2012). In the 1950s and 1960s, it was 'the lizard' in comparative physiological and anatomical studies (Dessauer, 1952; Licht & Rosenberg, 1969). It was also the first reptile to have its genome sequenced (Alföldi *et al.*, 2011; Losos *et al.*, 2005). Overall, an enormous body of research has been conducted on this species (Lovern, Holmes & Wade, 2004).

Despite its prevalence, and the great range of environments that it encounters through its range, to date very little research has examined morphological variation in this species. Dewlap coloration, behavioural display, cold tolerance, and egg size are among the few traits for which geographical variation has been previously documented (Crews, 1975; Wilson & Echternacht, 1987; Michaud & Echternacht, 1995; Macedonia, Echternacht & Walguarnery, 2003; Bloch & Irschick, 2006). Body and limb morphology too have been shown to differ significantly over distances as short as 30 km (Irschick *et al.*, 2005), although this type of variation has not been comprehensively assessed over the full distribution of *A. carolinensis*.

Studies on other Caribbean species have confirmed that anoles can differentiate over relatively short

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distances, adapting to variable environmental conditions (Losos, 2009). In the Lesser Antilles, for example, substantial within-island variation in body size, limb proportions, and scalation has been shown to correlate with rainfall, temperature, moisture, and vegetation (Lister, 1976; Malhotra & Thorpe, 1997b; Thorpe *et al.*, 2004; Calsbeek, Knouft & Smith, 2006; Wegener, Gartner & Losos, 2014). We set out to examine whether similar evidence of environmental adaptation exists in the green anole, which occupies a substantially greater diversity of environmental conditions.

The large geographical range of the green anole also makes possible an examination of the degree to which this species conforms to two well known eco-geographical trends: Bergmann's and Allen's rules. Bergmann's rule states that most endotherms exhibit a positive relationship of body size with latitude (Bergmann, 1847). Lizards, however, have been shown to reverse this rule, with larger body sizes in warmer, southern environments (Ashton & Feldman, 2003). This may be partly a result of the reliance of ectotherms on outside heat sources for increases in body temperature, which could confer an advantage for traits and behaviours that increase heat uptake in cold areas, such as the high surface area-to-volume ratios of smaller animals (Ashton & Feldman, 2003; Pörtner & Farrell, 2008; Alho *et al.*, 2011). The same mechanisms might lead us to predict a similar reversal of Allen's rule, which traditionally (as applied to endotherms) documents a decrease in appendage length in colder environments (Allen, 1877). Whether Allen's rule applies to ectotherms has been little investigated (Alho *et al.*, 2011).

To better document and understand geographical variation in morphology and its environmental correlates in *A. carolinensis*, we measured body traits and a suite of environmental parameters for 14 populations across the south-eastern USA. With these data, we aimed to build upon former studies of geographical variation in the green anole (Crews, 1975; Wilson & Echternacht, 1987; Michaud & Echternacht, 1995; Macedonia *et al.*, 2003; Bloch & Irschick, 2006). In particular, we set out to gain a clearer understanding of how body size and shape in male anoles are influenced by geographical variation in vegetation availability and abiotic environmental factors. Our study addresses three questions. (1) Which traits vary most among populations of male *A. carolinensis*, and how is variation distributed across the species' range? (2) Is variation in morphological traits correlated with environmental variation and, if so, how? (3) Do Bergmann's or Allen's rules apply to green anoles?

MATERIAL AND METHODS

COLLECTION OF MORPHOLOGICAL DATA AND ASSESSMENT OF VARIATION

Male *A. carolinensis* ($N = 115$) were collected from 14 locations across the species' range in the south-eastern USA. All specimens were collected in July and August of 2012, fixed in 70% ethanol, and accessioned into the Harvard MCZ collections. For each lizard, we recorded snout-vent length (SVL) and mass. Sites are mapped in Figure 1 (with sample sizes) and described in Table 1.

We X-rayed each specimen and used TPSDIG2 (<http://life.bio.sunysb.edu/morph>) to measure 26 limb, head (see Supporting information, Fig. S1), and body traits, many of which have been previously shown to be important for ecological adaptation in *Anolis* (Losos, 2009; Mahler *et al.*, 2010). Bilateral characters were measured on both sides of the animal and averaged. In each radiograph, we set the scale by recording the length of 1 cm on a millimeter ruler. We took triplicate measurements for a subset of our samples ($N = 14$) to allow assessment of repeatability with the ICC package for R, version 3.0.1 (Wolak, Fairbairn & Paulsen, 2012; R Core Team, 2013). Because our measurements were highly repeatable [$> 95\%$ intraclass correlation coefficient, with the exception of braincase width (approximately 86%) and metacarpal length (approximately 90%)], only data from the first set of measurements were retained for further analysis (Table 2). Data were then natural log-transformed. Residual values for each trait were calculated by regression against natural log-transformed SVL.

To reduce the dimensionality of the data, we performed a principal component analysis (PCA) on the full morphology dataset, from which individual values were then averaged to calculate mean population PC scores. We performed a series of analyses of variance (ANOVA) to examine morphological differentiation among populations in addition to hierarchical clustering analysis using the unweighted pair group method with arithmetic mean (Sokal & Michener, 1958). To test adherence to Allen's rule, we conducted another PCA on size-adjusted limb measurements alone. Similarly, to test for Bergmann's rule, we performed a PCA on SVL and mass and subsequently used the values on PC1 as a proxy for body size. For all PCAs, axes with an eigenvalue (SD^2) larger than 1 were retained for subsequent analyses (Jackson, 1993).

COLLECTION OF ENVIRONMENTAL DATA

We analyzed a suite of environmental variables for each of our collection sites (Table 3). Temperature

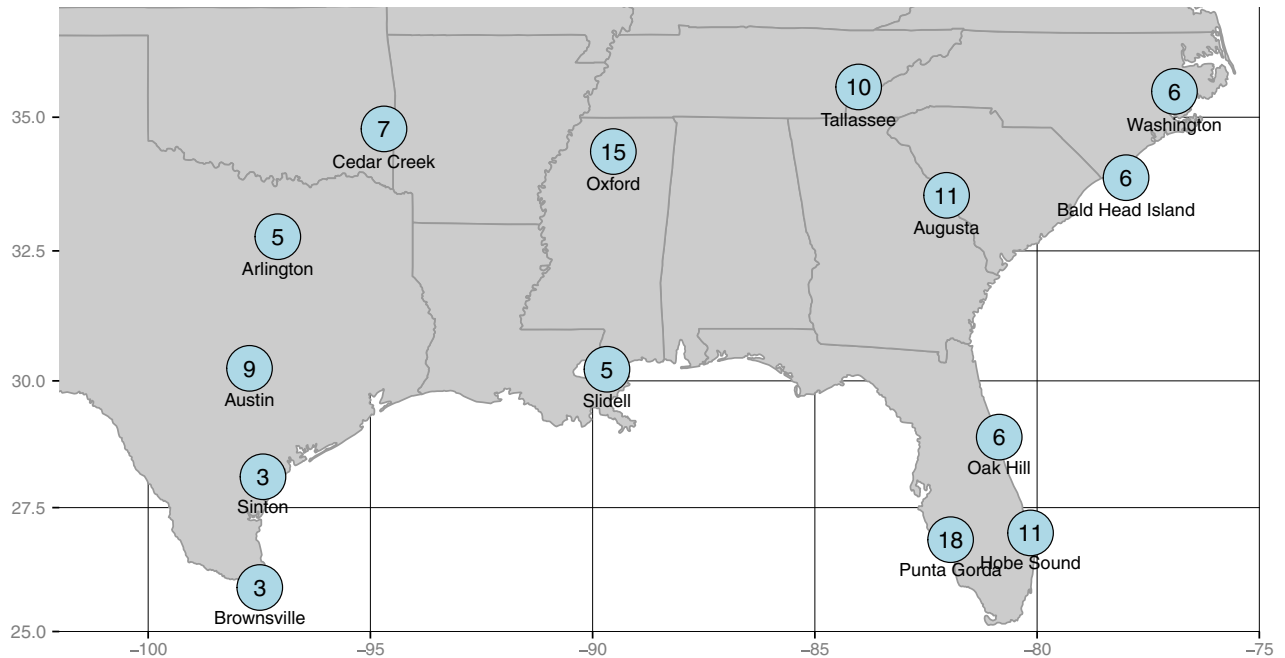


Figure 1. Density and distribution of sampling in the present study. Labels refer to the number of male *Anolis carolinensis* individuals collected at each site.

Table 1. List of collection sites with sample size and basic environmental data

City	State	Sample size	Longitude	Latitude	Annual mean temperature (°C)	Annual precipitation (mm)
Arlington	Texas	5	-97.082824	32.768435	18.6	877
Augusta	Georgia	11	-82.03618	33.548119	17.1	1158
Austin	Texas	9	-97.719787	30.243794	20.2	804
Bald Head Island	North Carolina	6	-77.998125	33.878317	17.4	1403
Brownsville	Texas	3	-97.488879	25.894197	23.2	688
Cedar Creek	Oklahoma	7	-94.694116	34.784428	15.4	1250
Hobe Sound	Florida	11	-80.147032	26.993678	23.3	1419
Oak Hill	Florida	6	-80.851844	28.898232	21.5	1300
Oxford	Mississippi	15	-89.534028	34.364995	15.8	1441
Punta Gorda	Florida	18	-81.946016	26.858265	22.9	1273
Sinton	Texas	3	-97.416889	28.113654	21.7	881
Slidell	Louisiana	5	-89.677461	30.226228	19.6	1529
Tallassee	Tennessee	10	-84.014709	35.556111	14.3	1376
Washington	North Carolina	6	-76.909324	35.474022	16.2	1298

and precipitation variables were taken from the WorldClim Bioclim database (<http://worldclim.org/bioclim>) and the vegetation variable Normalized Difference Vegetation Index (NDVI) was from the MODISTools R package (Tuck & Phillips, 2014). ‘Temperature seasonality’ was modified from Bioclim to represent the SD of temperature at a given

location. We ran separate PCAs on the temperature and precipitation variables to reduce dimensionality in each dataset. Again, axes with an eigenvalue larger than 1 were retained. We also retained annual mean temperature and NDVI separately and standardized them so they would be comparable to the PC axes.

Table 2. Intraclass correlation coefficient (ICC) (all $P \ll 0.05$), mean, SD, and principal component (PC) axis loadings/eigenvalues (SD^2) for each morphological trait

	Trait	ICC	Mean (mm)	SD	Full PC1	Full PC2	Full PC3	Full PC4	Full PC5	Full PC6	Limb PC1	Limb PC2	Size PC1
Hindlimb	Hindlimb phalanx	0.96	3.70	0.41	-0.16	0.20	-0.18	0.05	-0.19	0.00	0.32	0.23	-
	Hindlimb metatarsal	0.99	5.83	0.63	-0.16	0.27	-0.17	-0.01	-0.02	-0.14	0.36	0.00	-
	Tibia	0.99	9.42	1.06	-0.22	0.21	-0.15	-0.06	-0.20	0.35	0.37	0.34	-
	Fibula	0.99	9.28	1.08	-0.21	0.17	-0.18	-0.07	-0.16	0.39	0.35	0.34	-
	Femur	1.00	11.88	1.35	-0.18	0.27	-0.07	-0.09	-0.13	0.18	0.35	0.21	-
	Pelvis width	0.99	5.64	0.87	-0.03	0.23	-0.01	-0.14	0.30	-0.37	-	-	-
Forelimb	Metacarpal	0.90	2.36	0.32	-0.10	0.19	-0.02	-0.06	-0.06	-0.42	0.21	-0.08	-
	Ulna	0.99	6.69	0.82	-0.15	0.25	-0.16	0.13	0.43	-0.02	0.34	-0.53	-
	Radius	0.99	5.83	0.72	-0.13	0.21	-0.17	0.13	0.51	-0.03	0.30	-0.62	-
	Humerus	0.99	9.55	1.18	-0.20	0.21	-0.22	0.04	0.03	0.00	0.37	-0.03	-
Head	Opening inlever	0.97	2.11	0.37	0.00	0.06	-0.04	-0.47	-0.27	-0.44	-	-	-
	Closing inlever	0.97	3.64	0.52	-0.21	0.07	-0.07	0.20	-0.28	-0.14	-	-	-
	Whole head	1.00	17.96	2.26	-0.31	-0.12	0.04	-0.12	-0.08	-0.15	-	-	-
	Outlever	1.00	15.86	1.94	-0.32	-0.14	0.05	0.02	0.01	-0.02	-	-	-
	Snout plus eye	0.99	12.22	1.49	-0.26	-0.23	0.11	-0.12	0.19	0.07	-	-	-
	Snout length	0.98	6.55	0.87	-0.26	-0.20	0.10	0.24	-0.01	-0.06	-	-	-
	Real eye length	0.99	6.09	0.75	-0.03	-0.04	0.04	-0.64	0.30	0.24	-	-	-
	Braincase width	0.86	2.48	0.36	0.09	0.25	0.28	0.17	0.06	-0.06	-	-	-
	Head width retro.	1.00	8.43	1.19	0.02	0.24	0.38	0.03	-0.03	0.19	-	-	-
	Head width jugals	1.00	9.83	1.40	-0.04	0.26	0.37	-0.13	-0.06	0.07	-	-	-
	Head width quadrates	1.00	9.66	1.37	0.00	0.28	0.38	-0.07	-0.12	0.01	-	-	-
	Snout width	0.99	5.39	0.80	-0.07	0.15	0.42	0.13	0.08	-0.03	-	-	-
	Lower jaw length	1.00	18.45	2.31	-0.31	-0.09	0.09	-0.12	-0.07	-0.12	-	-	-
	Quadrate to symphysis	1.00	16.58	2.03	-0.32	-0.10	0.10	0.01	-0.01	-0.02	-	-	-
	Jugal to symphysis	0.99	13.17	1.62	-0.27	-0.18	0.17	-0.14	0.18	0.08	-	-	-
	Orbit to symphysis	0.99	7.08	0.94	-0.26	-0.17	0.16	0.25	0.01	-0.07	-	-	-
Size	Snout-vent length	0.99	59.21	7.35	-	-	-	-	-	-	-	-	-0.71
	Mass (g)	-	4.33	1.58	-	-	-	-	-	-	-	-	-0.71
	Eigenvalue (SD^2)	-	-	-	8.54	5.11	3.31	1.76	1.43	1.13	5.2	1.19	1.94

COLLECTION AND ANALYSIS OF GENETIC DATA

The importance of incorporating phylogenetic information in comparative analyses has been noted in the literature (Felsenstein, 1985; Cruz *et al.*, 2005; Pincheira-Donoso, Hodgson & Tregenza, 2008). To account for shared ancestry and ongoing gene flow in the present study, we assembled genetic data from two mitochondrial (ND2 and tRNAs) and three nuclear genes (TERT, RALGAPA and HMGCS) from Genbank records for three previous studies (Campbell-Staton *et al.*, 2012; Tollis *et al.*, 2012; Tollis & Boissinot, 2014). Our localities were matched with the closest genetic site from these three studies (see Supporting information, Fig. S2, Table S1). Sites for which there were no matching genetic samples within 250 km were

excluded from these analyses. The final mitochondrial (mt)DNA dataset included 76 sequences, with all 14 populations within 250 km of at least one genetic sampling locality. The nuclear (n)DNA dataset, concatenated across the three nuclear genes, included 43 sequences that were proximate to 11 of our populations. Sequences were aligned in CLUSTALW2, version 2.1 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>), and imported to MESQUITE, version 2.75 (Maddison & Maddison, 2011). We then calculated genetic divergence between localities using a basic substitution model (K80) determined by JMODELTEST, version 2.1.6 (Darriba *et al.*, 2012). Finally, we converted these into matrices of pairwise genetic distance between sites (see Supporting information, Tables S2, S3).

Table 3. Loadings of environmental variables on temperature and precipitation principal component (PC) axes, with their eigenvalues (SD^2)

	Variable	Temperature PC1	Temperature PC2	Precipitation PC1	Precipitation PC2
Temperature	Annual mean temperature	0.362	-0.125	-	-
	Mean diurnal range	-0.322	0.047	-	-
	Isothermality	0.267	0.327	-	-
	Temperature seasonality	-0.354	-0.174	-	-
	Maximum temperature warmest month	0.058	-0.584	-	-
	Minimum temperature coldest month	0.371	0.011	-	-
	Temperature annual range	-0.357	-0.172	-	-
	Mean temperature wettest quarter	0.289	-0.224	-	-
	Mean temperature driest quarter	0.131	0.465	-	-
	Mean temperature warmest quarter	0.251	-0.455	-	-
	Mean temperature coldest quarter	0.369	-0.007	-	-
Precip.	Annual precipitation	-	-	0.452	-0.109
	Precipitation wettest month	-	-	0.168	-0.52
	Precipitation driest month	-	-	0.427	0.182
	Precipitation seasonality	-	-	-0.271	-0.457
	Precipitation wettest quarter	-	-	0.288	-0.444
	Precipitation driest quarter	-	-	0.427	0.201
	Precipitation warmest quarter	-	-	0.294	-0.419
	Precipitation coldest quarter	-	-	0.401	0.249
	Eigenvalue (SD^2)	7.25	2.32	4.67	3.01

REGRESSION AND ENVIRONMENTAL CORRELATION

To assess the degree to which morphological and environmental variables were correlated, we ran a series of mixed-model univariate regressions with the MCMCglmm package in R (Hadfield, 2010; Stone, Nee & Felsenstein, 2011). Beginning with the full morphology dataset, we tested the correlation of the six significant PCAs with temperature, precipitation, and vegetation. The appropriate genetic distance matrix was decomposed with the svd function in R, and then used in the regression as a random effect to account for shared ancestry and gene flow. We used the default number of iterations (13 000) and burn-in (3000) in the estimation of parameters, as well as a flat/non-informative prior. These analyses were run separately for the mtDNA and nDNA matrices. To investigate the adherence of *A. carolinensis* to Allen's rule, we repeated this procedure for the significant limb PCAs, regressing them against the temperature variables (including annual mean temperature alone) in addition to latitude. For Bergmann's rule, we tested correlation of temperature and latitude with two measures of size: the first PCA of the size variables, as well as the log-transformed SVL. Given that the environmental variables that we examined are strongly and linearly related to geography, we did not correct for spatial autocorrelation, *sensu* Hawkins (2012).

To make our results reproducible, and to aid in future studies of geographical variation, we have made our data and scripts available in their entirety online (<https://github.com/alj0217/carolinensis>). In addition, all computational tools used in the analytical portion of the present study (R and its packages) are free and open source.

RESULTS

GEOGRAPHICAL VARIATION IN MORPHOLOGY

Most traits scaled tightly ($r^2 > 0.80$, $P < 0.05$) with SVL (see Supporting information, Fig. S3, which depicts femur length), although braincase width in particular (see Supporting information, Fig. S3) was substantially more variable ($r^2 = 0.10$, $P < 0.05$). Residual values for other traits also showed considerable variation among populations (see Supporting information, Fig. S4).

We retained six axes from the PCA on all morphological characters that explained $> 80\%$ of the variation. PC1 loaded most heavily on head length traits, PC2 on head width traits and hindlimb traits, PC3 on head width traits, PC4 on head length traits, PC5 on forelimb traits, and PC6 on a head length trait and several limb traits (Table 2). The analysis of limb traits for the test of Allen's

rule produced two significant PC axes explaining over 70% of variation, with the first loading most heavily on humerus and tibia lengths and the second on radius and ulna lengths. Finally, the analysis of SVL and mass for Bergmann's rule yielded one significant component explaining over 96% of the variation, loading equally on the two original variables. Figure 2 provides a graphical summary of PC scores for these three analyses, also showing considerable intrapopulation variation for some sites.

In the full dataset PCA, all six axes differed significantly among populations (ANOVA, $P < 0.05$ in all cases). A hierarchical clustering analysis based on these axes produced groupings that did not always correspond with geography (Fig. 3). Most notable were two clusters containing southern anoles from opposite ends of the Gulf of Mexico: one contained the Oak Hill (FL) and Sinton (TX) populations and the second contained those from Brownsville (TX) and Hobe Sound (FL). We also observed clustering of northern and southern populations, with the latter

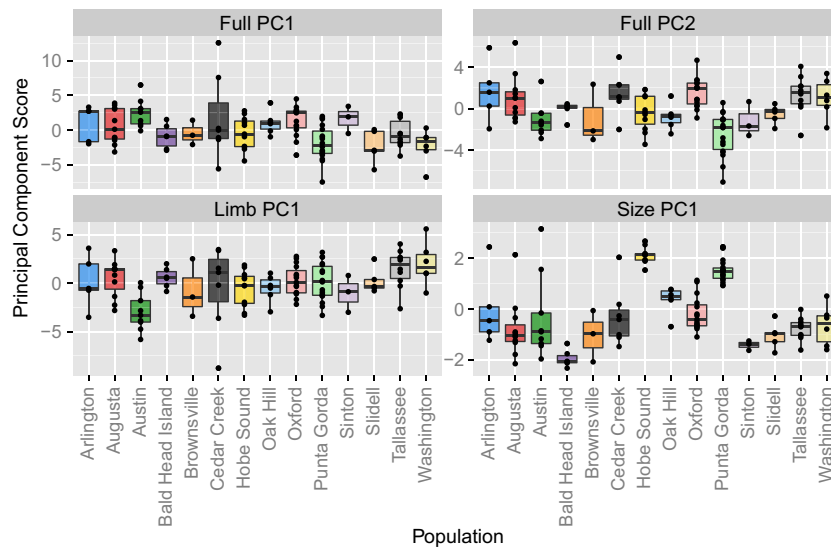


Figure 2. Full morphology, limb trait, and size principal component scores by population.

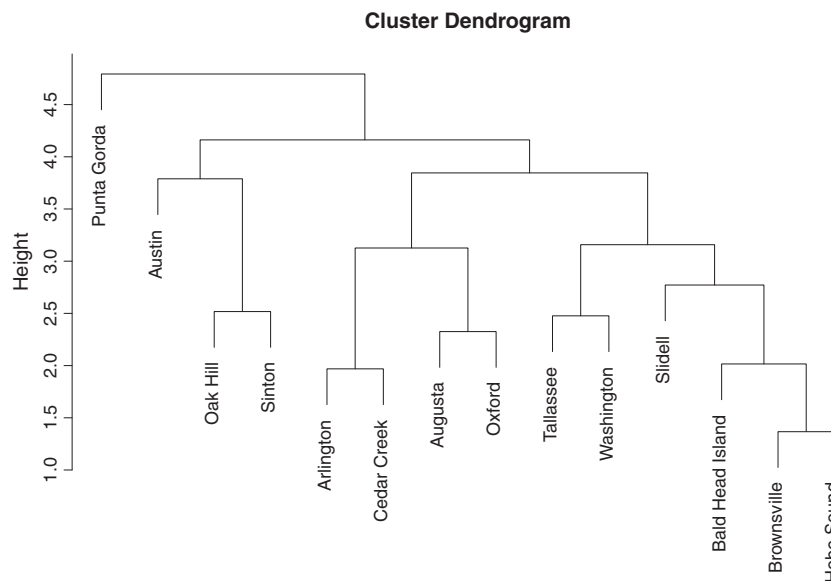


Figure 3. Hierarchical clustering of populations based on full morphology principal component scores.

TX/FL pair grouped with Bald Head Island, Tallassee, and Washington. However, we also recovered some clustering of geographically proximal sites, including Arlington and Cedar Creek. Anoles from these two sites were part of a larger northern grouping of four populations alongside Augusta and Oxford. Punta Gorda was highly distinct, distant to all other populations.

Plots of population-average PC scores corroborated some of these findings from the clustering analyses. The plot of full morphology PCs 1–2 again shows some support for the grouping of south Texan and Floridian anoles, as well as the association among the four northern populations mentioned above (Fig. 4). Based on full morphology PC axes, Punta Gorda, Slidell, and Washington anoles appeared to be unique, and separate from the majority of other populations.

ENVIRONMENTAL CORRELATION

The PCA on temperature-related climate variables revealed two primary axes accounting for over 85% of the variation, with PC1 loading most heavily on cold extremes and PC2 on warm extremes and temperature in the driest quarter. The PCA on precipitation variables resulted in two axes explaining over 95% of variation in these data, with the first loading most heavily upon annual precipitation level and the second on precipitation level in the wettest month (Table 3).

We did not find any significant associations of environment with the first, third, fourth or sixth principal component of full morphology. For the second PC, however, latitude, temperature, and precipitation (PC2) were highly significant predictors of

axis score ($P < 0.05$). The fifth morphology PC also showed a significant relationship with temperature. The vegetation variable (NDVI) did not correlate with any of the morphology PC axes. In the test of Allen's rule, latitude and temperature correlated with the limb morphology axes. However, we found no support for Bergmann's rule in that neither overall size, nor SVL associated significantly with temperature or latitude.

In general, the nDNA matrix did not differ greatly from mtDNA matrix as a 'random effect' in these regressions. Although mitochondrial and nuclear genes can sometimes yield conflicting pictures of evolution (Shaw, 2002; Spinks & Shaffer, 2009), the few differences that we did observe were likely attributable to incompleteness of the nDNA data, which contained only 11 out of 14 populations. As such, associations under the mtDNA dataset are reported in Table 4 and plotted in Figure 5 with regression lines inferred by the MCMC algorithm. Regressions under the nDNA dataset are reported in the Supporting information (Table S4).

DISCUSSION

Anolis carolinensis has long served as a model organism for anatomy and physiology, and has great potential to do the same for studies of geographical differentiation and adaptation. Variation in morphology has been well documented in island anole species across smaller geographical and environmental ranges; however, much less is known about broadly distributed mainland anoles such as *A. carolinensis*. Remarkably, this species occurs from the subtropical regions of southern Florida to Oklahoma, where

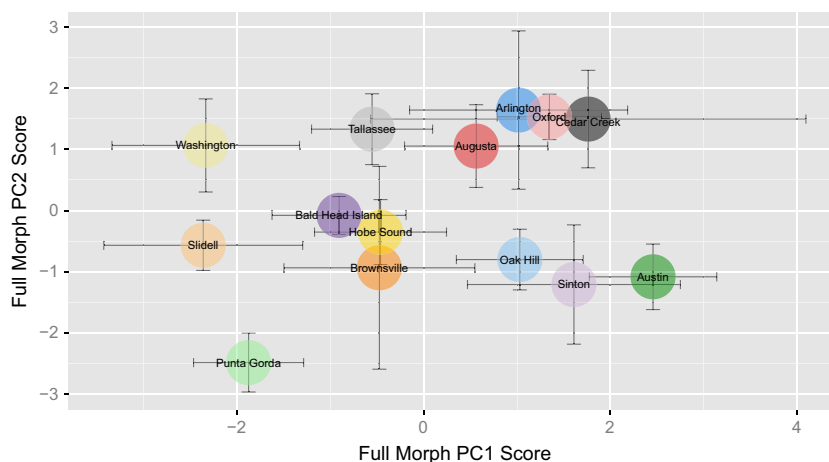


Figure 4. Clustering of population principal component scores, represented as means among all individuals from that site. Bars represent the SEM.

Table 4. Posterior mean intercept, mean and bounds of coefficient, and *P*-value for significant regressions between environment and morphology (mitochondrial DNA)

Formula	Posterior mean intercept	Posterior mean coefficient	Coefficient 95% lower	Coefficient 95% upper	<i>P</i>	Confirmed by nuclear DNA
Full PC2 – Temperature PC1	0.013	–0.409	–0.575	–0.220	< 0.001	X
Full PC2 – Annual temperature	0.048	–1.092	–1.622	–0.677	< 0.001	X
Full PC2 – Precipitation PC2	0.028	0.561	0.159	1.018	0.006	X
Full PC2 – Latitude	–9.884	0.318	0.185	0.438	< 0.001	X
Full PC5 – Temperature PC1	–0.116	–0.169	–0.324	–0.022	0.032	
Limb PC1 – Temperature PC2	–0.019	0.462	0.052	0.836	0.028	
Limb PC1 – Annual temperature	–0.118	–0.720	–1.358	–0.118	0.026	X
Limb PC1 – Latitude	–6.450	0.204	0.011	0.381	0.032	X
Limb PC2 – Temperature PC1	0.124	0.145	0.001	0.266	0.022	

PC, principal component.

winters bring regular snowfall and temperatures below freezing. Based on evidence of adaptation to environmental conditions in other anoles with far less climatically diverse ranges, we predicted that *A. carolinensis* would exhibit a high degree of morphological variation and that this variation would be related to environment across this species' range.

PATTERNS OF GEOGRAPHICAL VARIATION

As hypothesized, we found significant geographical variation in morphology among populations sampled in the present study. Although most within-trait differences in the raw data were accounted for by size (SVL), substantial residual variation existed for many measured characteristics. Residual variation in braincase width (the highest of all traits), however, may be partially attributable to lower measurement repeatability. PCA loadings indicated that head shape (a combination of length and width) was the most important driver of geographical differentiation in this species.

Given this, head width and length played a significant role in distinguishing certain populations as unique. For example, Punta Gorda anoles have very narrow but long heads after controlling for body size. This unique head shape distinguished lizards of that locality from other populations, as revealed by their extreme position in the hierarchical clustering and PC space analyses (Figs 3, 4). Figure 6 contrasts radiographs at similar magnification of individuals from Cedar Creek and Punta Gorda, demonstrating the special characteristics of Punta Gorda anoles, as well as the overall degree of head shape variation in our dataset.

Variation in head shape has been demonstrated in studies of several lizard species to be related to bite force, jaw speed, and foraging strategy, which in

turn correlate with type and hardness of prey (Herrel *et al.*, 2006; Herrel, McBrayer & Larson, 2007; McBrayer & Corbin, 2007). The morphology of Punta Gorda and Slidell anoles, both of which had long and narrow heads for their size, may suggest that these lizards can close their jaws more rapidly, aiding in the capture of more evasive prey or the processing of smaller but more numerous prey items (Herrel *et al.*, 2007; McBrayer & Corbin, 2007). Bite force has also been shown to play a role in mediating territorial conflicts among males (Lailvaux *et al.*, 2004), indicating that male–male combat may be another driver of differences in head shape among these populations. Additional studies of geographical variation in prey diversity, male density, and combat frequency would help to understand the potential drivers of the head shape variation observed here.

We also identified unique populations based on significant limb variation; for example, lizards from Austin had substantially shorter limb lengths relative to body size than lizards from other sites. Indeed, previous work has demonstrated a relationship between hindlimb dimensions and perch use, with limb length being correlated with perch diameter (Losos, Warheit & Schoener, 1997; Malhotra & Thorpe, 1997a; Losos *et al.*, 2001; Calsbeek, Smith & Bardeleben, 2007). The anomalous limb characteristics of Austin anoles warrant further investigation of the particular vegetative microhabitats that characterize that site.

Despite the unique head and limb morphology of some anoles, clustering of populations by PC score indicated the potential for morphological convergence over geographical space in male *A. carolinensis*. Previous work has examined the relationship of morphology and environment in this species; most notably, Michaud & Echternacht (1995), in a study that focused on females, discovered relationships of body

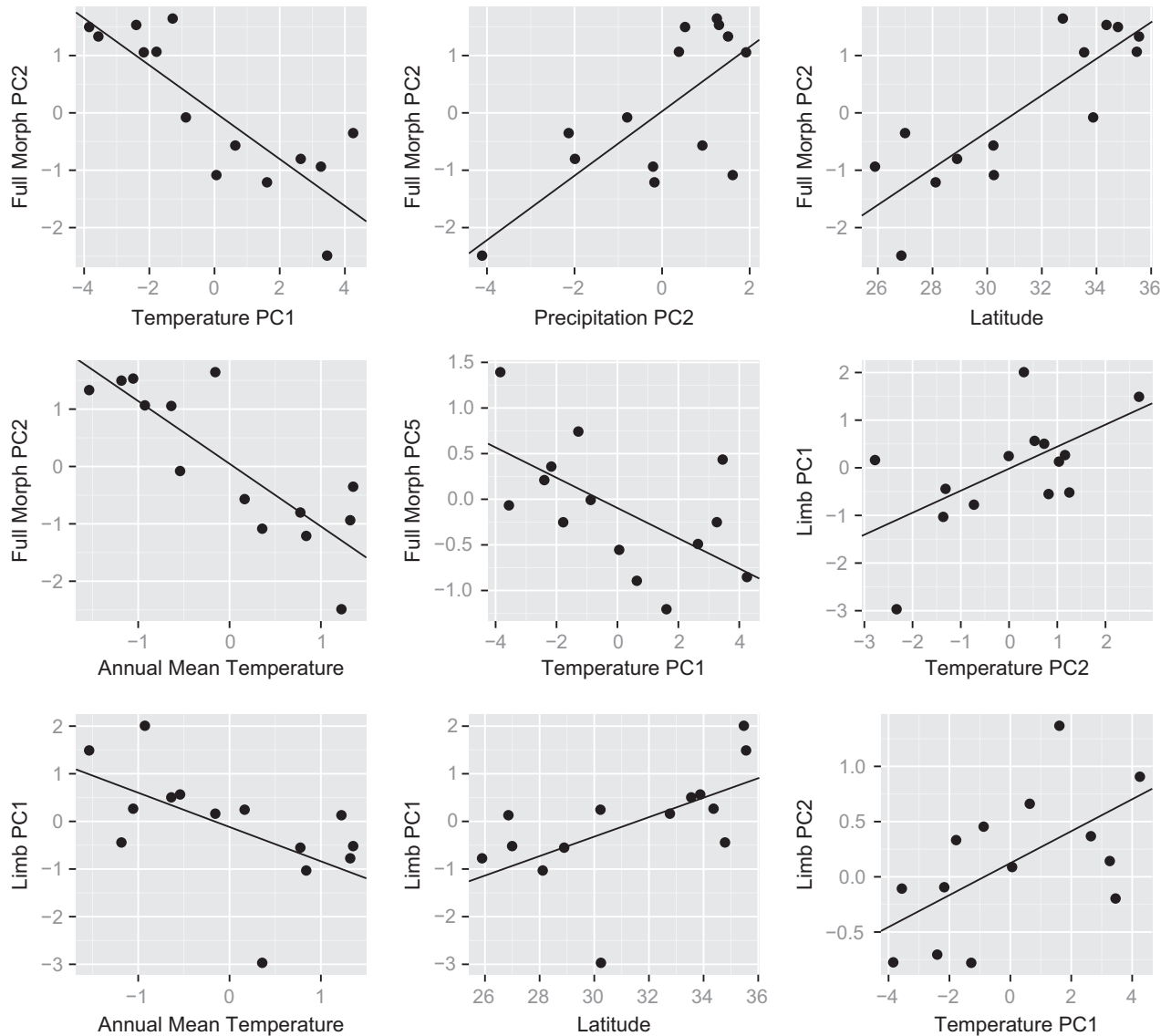


Figure 5. Summary of statistically significant correlations between environment and morphology.

size, egg size, and latitude. Our work expands on their study by testing a comprehensive set of environmental factors that may help to explain similarities among nonproximal male populations.

GENERAL ENVIRONMENTAL CORRELATION OF *A. CAROLINENSIS* MORPHOLOGY

Links between morphology and environment in anoles are well documented in the literature. Humidity, rainfall, temperature, and vegetation can all be predictors of phenotype (Malhotra & Thorpe, 1997a; Losos, 2009): scalation is often related to temperature, moisture, and vegetation (Lister, 1976; Malhotra & Thorpe, 1997b; Thorpe *et al.*, 2004;

Calsbeek *et al.*, 2006; Wegener *et al.*, 2014), whereas body dimensions have been shown to correspond to features of vegetation and altitude (Losos *et al.*, 1997, 2001; Malhotra & Thorpe, 1997a; Calsbeek *et al.*, 2007). To date, however, these studies have been mainly conducted on island species. In the present study, we examined variation in a widespread mainland anole to test for some of these same correlations.

We report novel relationships of temperature, latitude, and precipitation with anole head and limb morphology. Looking specifically at the second morphological PC axis, we found that anoles in more seasonal and colder climates of the north tended to have to have relatively longer limbs and wider and

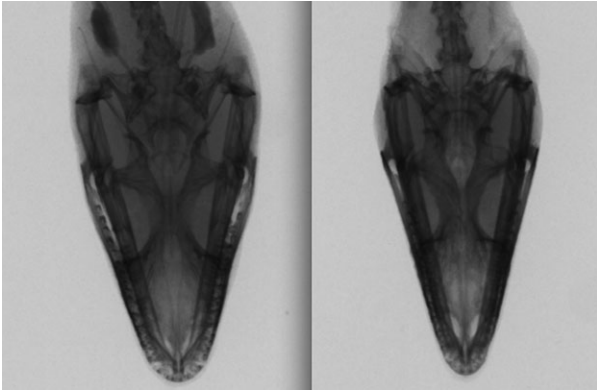


Figure 6. Head shape variation between an anole from Cedar Creek, OK (left) and one from Punta Gorda, FL (right).

shorter heads than those from less seasonal/warmer locations in the south (Figure 7). The relationship of temperature with the fifth PC axis partially confirms these findings, although it was more difficult to interpret biologically. These correlations raise questions about the functional relationships among these variables, which have not been widely studied in this species. Given the association of head traits with diet, our analysis also warrants further exploration of relationships between food source and other environmental parameters. Precipitation, too, was a significant predictor of morphology; we also found evidence for longer limbs and wider heads in populations whose sites were rainier during the coldest and driest quarters of the year. These environments are typical of the northern range of *A. carolinensis*, and thus corroborate the evidence from the analyses of temperature and latitude.

Although temperature, latitude, and precipitation correlated with morphology, vegetation did not show any significant associations in our analyses. As noted above, previous studies have reported a relationship between limb length and perch width for a variety of anole species. Our vegetation dataset, however, was composed solely of NDVI, which is a satellite measure of green leaf photosynthetic density in a terrestrial area. As a metric of macroscale climate differentiation, NDVI alone captures relatively little of the micro-structural habitat previously associated with divergence in morphology. However, this macro-structural characteristic could conceivably determine the entire distribution of available perches, with potential ramifications for niche partitioning and adaptation. Incorporation of additional variables, especially those measuring perch characteristics, might help to clarify the relationship of vegetation and morphology in *A. carolinensis*.

TESTING REVERSE BERGMANN'S RULE IN *A. CAROLINENSIS*

Although controversial (Pincheira-Donoso *et al.*, 2008), meta-analytic approaches for assessing body size gradients have revealed general evidence for reverse Bergmann's rule in squamates (Ashton & Feldman, 2003). This means, contrary to the rule's original formulation for endotherms, that most squamate species show decreasing body size with increasing latitude and decreasing temperature (Bergmann, 1847; Ashton & Feldman, 2003). Among endotherms, a larger size confers a smaller surface area-to-volume ratio that may help to limit heat loss in cold environments (Bergmann, 1847). Ectotherms, on the other hand, achieve increases in body temperature not by internal production, but by exposure to outside heat sources (Alho *et al.*, 2011). As a result, high surface area-to-volume ratios associated with small sizes may be beneficial for these animals, allowing a more rapid uptake of heat in cold environments (Ashton & Feldman, 2003).

These data did not support our hypothesis of reverse Bergmann's rule in *A. carolinensis*; the pattern of body size variation seen in the present study and another on the same species (Goodman *et al.*, 2013) suggests that the explanation may be more complex. Although somewhat noisy, body size in Floridian populations appeared to decrease with temperature, whereas the relationship was reversed among non-Floridian ones (see Supporting information, Fig. S5). These conflicting patterns highlight the need for further studies of Floridian populations, where this trait might be under a more complex set of selective pressures.

Our results disagree with those of Michaud & Echternacht (1995), who found a trend of increasing body size with latitude among female *A. carolinensis*. This discrepancy may partly be the result of selective forces specific to females, such as an advantage to producing larger juveniles in northern populations (Michaud & Echternacht, 1995). However, further studies are needed to understand geographical variation in sex-specific selective pressures in this species and the patterns of size and shape dimorphism that may result.

EVIDENCE FOR REVERSE ALLEN'S RULE

Allen's rule states that animals from colder, northern climates tend to have relatively shorter appendages than those from warmer environments farther south (Allen, 1877). To date, this rule has been reported primarily for endotherms, and its traditional explanation accords with that for Bergmann's rule: shorter appendages in colder environments minimize heat loss by

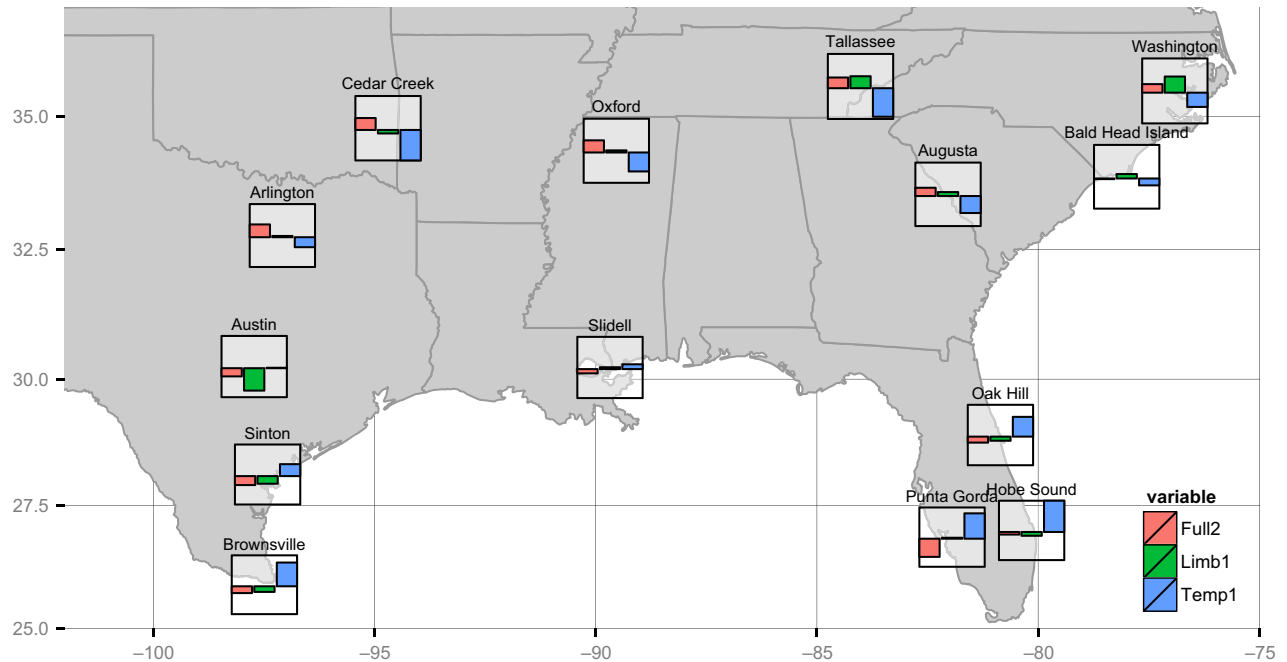


Figure 7. Geographical distribution of principal component (PC) scores showing the relationship between morphology and temperature. Represented are the second axis of the full morphology PC analysis (Full2), the first axis of the limb PC analysis (Limb1), and the first axis of the temperature PC analysis (Temp1).

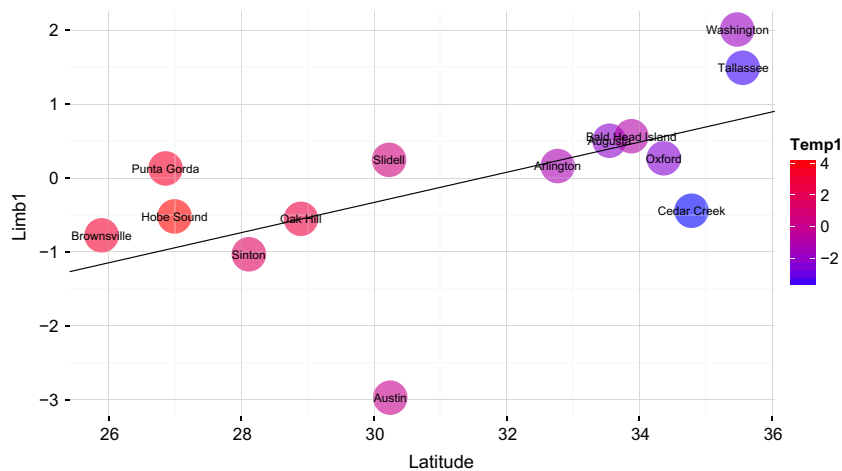


Figure 8. Latitude, temperature, and morphology interaction for the first component of the limb principal component analysis, providing a graphical summary of the evidence for Reverse Allen's rule in this species.

decreasing the surface area to volume ratio (Allen, 1877; Alho *et al.*, 2011). In ectotherms, we might predict that, analogous to explanations for Reversed Bergmann's rule, appendage length would increase in colder climates. However, previous studies of limb length over environmental gradients in ectotherms are generally inconclusive, with some supporting Allen's rule (Ray, 1960) and others supporting its converse (Bidau & Martí, 2008; Langkilde, 2009).

Unlike body size, for which we saw no appreciable trend, limb dimensions in *A. carolinensis* did show evidence for reverse Allen's rule. Our analyses revealed significant correlations of limb morphology with both latitude and the second principal component of temperature, indicating that lizards at northern sites with lower maximum/annual temperatures had longer limbs for their size than lizards at southern ones (Fig. 8). Although the second temperature

axis is slightly difficult to interpret geographically, the correlation of limb morphology with annual mean temperature lends an overall picture of reverse Allen's rule in this species. This trend may also help to explain the previously observed clustering of Floridian and Texan anoles, which are geographically distant but reside within a degree of latitude of one another. Ultimately, however, examination of other variables such as microhabitat and behaviour may be necessary to obtain a full picture of selection on hindlimb morphology in this species.

VARIATION: THE RESULT OF GENETIC CHANGES OR PHENOTYPIC PLASTICITY?

Correlations between geographical variation in morphology and environment could be the result of two factors: genetic changes driven by natural selection or, alternatively, phenotypic plasticity in which lizards growing up in different environments develop different phenotypes. Previous work has reported evidence for plasticity in hindlimbs that are affected by the size of the perches used by lizards during development in the laboratory (Losos *et al.*, 2000; Kolbe & Losos, 2005). Similarly, some morphological characters in an Australian lizard appear to be influenced by nest temperature (Qualls & Shine, 1998). However, because many of these previous studies were completed in confined conditions, the degree to which plasticity explains limb length variation among natural populations is not well known (Losos, 2009).

On the other hand, in a study of *A. oculatus* on the island of Dominica, Thorpe, Reardon & Malhotra (2005) found that population differences in limb, toe, head length, and scalation were retained when lizards were raised in a common garden, suggesting that geographical variation in these traits was genetically based. For the most part, however, the genetic underpinnings of geographically varying traits in anoles, and lizards in general, have not been well studied. In light of the considerable environmental variation and associated selective pressures across its distribution, variation in *A. carolinensis* may have both a plastic and a genetic component. Further studies are necessary to determine the relative contribution of these two forces in shaping morphology in this species.

CONCLUSIONS

The patterns of variation and environmental correlation that we report in the present study help to establish *A. carolinensis* as a strong candidate for further studies of morphological variation over a large range. Future work should focus on intraspecific

selection among populations and functional analysis of the environmental correlates identified in the present study, with greater attention given to their ability to drive differences in morphology. The recent publication of this species' genome may also allow for future investigation of the genetic bases behind this variation, as well as a better understanding of how populations are adapting and potentially diverging. Because phenotypic plasticity has recently been shown to play an important role in response to climate change (Chown *et al.*, 2007; Charmantier *et al.*, 2008), increased knowledge of the relationships between anole morphology and environmental parameters could in turn help to illuminate their adaptive potential in the face of habitat shift.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Head width (top) and length (bottom) traits measured in the present study. For ease of display, some head length traits are represented as a sum of smaller segments. (1) Head width, retroarticulars; (2) head width, quadrates; (3) braincase width; (4) head width, jugals; (5) snout width; (6) eye length; (7) orbit to

symphysis length; (8) jugal to symphysis length; (9) snout length; (9 + 10) snout + eye length; (11) closing inlever length; (9 + 10 + 11) outlever length; (12) closing inlever length; (9 + 10 + 11 + 12) whole head length; (13) lower jaw length; (14) quadrate to symphysis length.

Figure S2. Sites in the present study ('Our Morph Data') plotted with those from the three other studies from which genetic data were drawn.

Figure S3. Relationship of snout–vent length with femur length (A) and braincase width (B), which showed high variability. Traits are reported as natural log mm.

Figure S4. Residual values by population for the 26 head and body traits measured from radiographs in the present study.

Figure S5. Patterns of size variation in Floridian and non-Floridian anoles.

Table S1. Genetic data source sites for each morphological population collected in the present study.

Table S2. Mitochondrial genetic distance matrix used in the MCMCglmm regressions.

Table S3. Nuclear genetic distance matrix used in the MCMCglmm regressions.

Table S4. Posterior mean intercept, mean and bounds of coefficient, and *P*-value for significant regressions between environment and morphology (nuclear DNA).