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ORIGINAL ARTICLE

Influence of geography and climate on patterns of cell size and body size in the lizard *Anolis carolinensis*

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Abstract

Geographic patterns in body size are often associated with latitude, elevation, or environmental and climatic variables. This study investigated patterns of body size and cell size of the green anole lizard, *Anolis carolinensis*, and potential associations with geography or climatic variables. Lizards were sampled from 19 populations across the native range, and body size, red blood cell size and size and number of muscle cells were measured. Climatic data from local weather stations and latitude and longitude were entered into model selection with Akaike's information criterion to explain patterns in cell and body sizes. Climatic variables did not drive any major patterns in cell size or body size; rather, latitude and longitude were the best predictors of cell and body size. In general, smaller body and cell sizes in Florida anoles drove geographic patterns in *A. carolinensis*. Small size in Florida may be attributable to the geological history of the peninsular state or the unique ecological factors in this area, including a recently introduced congener. In contrast to previous studies, we found that *A. carolinensis* does not follow Bergmann's rule when the influence of Florida is excluded. Rather, the opposite pattern of larger lizards in southern populations is evident in the absence of Florida populations, and mirrors the general pattern in squamates. Muscle cell size was negatively related to latitude and red blood cell size showed no latitudinal trend outside of Florida. Different patterns in the sizes of the 2 cell types confirm the importance of examining multiple cell types when studying geographic variation in cell size.

Key words: Bergmann's rule, ecogeographic pattern, latitude, longitude, reptile, temperature

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INTRODUCTION

Biologists have long been fascinated with ecogeographic patterns in body size, such as interspecific and intraspecific trends in body size associated with latitude, elevation, or environmental and climatic variables. The well-known Bergmann's rule highlights the tendency for larger-bodied endothermic vertebrates to occur in

cooler climates (Bergmann 1847). The rule was originally intended to apply and has been tested at the interspecific level, but was refined and is generally applied at the intraspecific level (Mayr 1956; Blackburn *et al.* 1999; Ashton *et al.* 2000; Ashton 2002a). Explanations for larger size in endotherms at higher latitudes include fasting endurance through long winters and, Bergmann's original suggestion, that minimization of surface area relative to volume reduces heat loss (reviewed in Blackburn *et al.* 1999; Watt *et al.* 2010). Among ectothermic vertebrates, the Bergmann's heat conservation explanation does not apply as simply, because heating and cooling rates both vary with body size; in fact, intraspecific patterns of body size with latitude and temperature vary across ectothermic taxa (Watt *et al.* 2010). Studies of amphibians and fishes produce mixed results, with some taxa but not others conforming to Bergmann's rule (e.g. Power & McKinley 1997; Ashton 2002b; Belk & Houston 2002; Adams & Church 2008). Among reptiles, body size in turtles generally increases intraspecifically with increasing latitude and decreasing temperature, while lizards and snakes generally show opposing trends (reviewed in Watt *et al.* 2010). Ashton and Feldman (2003) suggest that smaller body size may be advantageous for rapid heating in squamates at higher latitudes. However, no generalizations of mechanisms for body size variation in squamates are currently accepted.

Many environmental variables are associated with latitude and longitude and may contribute to ecogeographic patterns. In addition to temperature, environmental moisture may also affect body size through associations with thermoregulation or environmental primary production and food availability (Yom-Tov & Geffen 2006). James (1970) found that smaller body size in North American bird populations was associated with both warmer temperatures and drier climates. In that study, large bodies were thought to be at a disadvantage in warm, moist environments because of high heat production (a consequence of larger body size) and low evaporative cooling potential. In non-avian reptiles, this explanation would not apply. However, environmental moisture is potentially influential in these taxa because body size exhibits plasticity with respect to developmental moisture levels in many species (reviewed in Packard & Packard 1988 and in Shine 2004).

Ecogeographic trends in cell size have also been documented in several ectotherms, although most research in this area has focused on invertebrates. Experimental studies in vertebrate ectotherms (mostly fishes) and invertebrates have demonstrated that colder temperatures result in larger animals composed of larger cells (Arendt 2007). This developmental response has been proposed to account for geographic trends in cell size and body size (Van Voorhies 1996). This hypothesis is, however, currently viewed as largely heuristic. The evolutionary advantage of larger cells at colder temperatures has been attributed to greater efficiency of larger cells with respect to energy (Szarski 1983; West *et al.* 2002). Animals composed of larger cells should have relatively lower metabolic rates, which may be advantageous in environments with lower resource availability (Szarski 1983; Kozlowski *et al.* 2003). In contrast, smaller cells have a higher metabolic rate and should be able to divide more quickly, leading to faster development (Szarski 1985). Van der Have and de Jong (1996) suggest that cell division and corresponding organismal maturation proceed faster than cellular growth as temperature increases, resulting in smaller adults composed of smaller cells at higher temperatures.

Trends in cell size and number may vary depending on the type of cell examined; therefore, use of multiple cell types is ideal. Red blood cells (RBCs), which have been used in several studies, are fully differentiated, uniform in shape, and do not enter cellular division (Starostova *et al.* 2005). However, RBCs are not structural tissues and they have a short life span (approximately 4 months) and so may acclimate more readily than other tissues. In several bird species, RBC size correlates with cell sizes in other tissues (Gregory 2000). However, consistency of cell size in different tissues varies taxonomically (Szarski 1985; Kozlowski *et al.* 2010). In addition, temperature may affect cell types differently (e.g. actively dividing cells *vs* differentiated cells) (Cuadrado *et al.* 1989; Atkinson 1994). Muscle cell (MC) size and number have been examined in temperature plasticity experiments in tadpoles and fish (reviewed in Arendt 2007). Ontogenetic effects must be considered when using these cells to study growth and development, because temperature may change patterns of muscle recruitment and development in addition to growth (Arendt 2000; Arendt & Hoang 2005).

The green anole, *Anolis carolinensis* Voigt, 1832 (Polychrotidae), is a small diurnal, arboreal lizard found in 11 states in southeastern USA (see Fig. 1a for range map and study site locations). Geographic trends in body size and cell size corresponding to Bergmann's rule (larger in the north) have been proposed (Goodman & Heah 2010), but sampling has not yet covered the entire species range. Multiple habitat types are occupied by this species throughout its range, which covers ap-

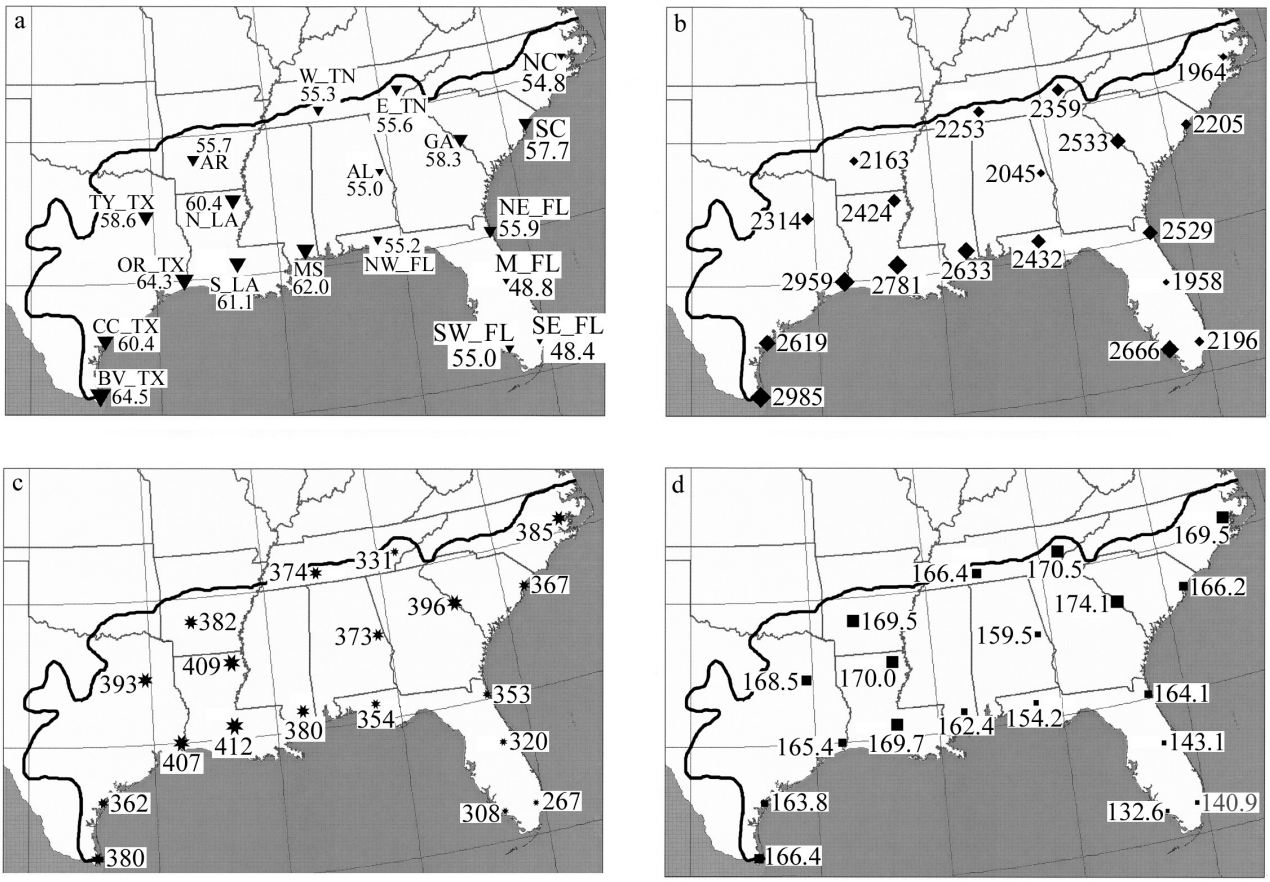

Figure 1 Range map of *Anolis carolinensis*, showing locations of 19 populations sampled in the current study. The bolded line shows the outer limits of the geographic range (based on map in Conant and Collins 1998). (a) Average SVL (cm) for each population, with triangles proportional in size to relative body size. (b) Average MC size (μm^2) with diamonds proportional in size to cell size. (c) Average MC number in 4 segments of skeletal muscle (viewed in cross-section) from tails, with stars proportional in size to cell number for each population. (d) Average RBC size (μm^2), with squares representative of cell size for each population.

proximately 22° longitude and 10° latitude. Turnover rates within populations are very high each year (estimates of >90 to 98%; Gordon 1956; King 1966; Michael 1972), indicating that few individuals survive for more than 1 reproductive season. Green anoles are active to some extent throughout the year and never enter full hibernation, even in areas where populations experience cold winters. In a northern population in Tennessee, individuals are active on warm, south-facing rock slopes on sunny days when ambient temperatures away from the slopes are below freezing (Bishop & Echternacht 2004).

Anolis carolinensis has a seasonal reproductive cycle; mating occurs in Apr or May through Jul or Aug, with some variation among populations (reviewed in Minesky 1999). Green anoles exhibit sexual size dimorphism, and geographic variation in this character has been documented by Goodman and Luck (unpubl. data). A recent study indicated a trend of increasing body size and larger RBC size with increasing latitude, but sampling was limited to females from 4 populations (Goodman & Heah 2010). The goals of the current study were to determine the patterns of average body size and cell size across the entire geographic range of the species and to determine whether the observed patterns might be associated with patterns of geographic variation in climate, longitude and latitude. Adults were collected at the beginning of the mating season, when they are ac-

tive and readily captured, when most individuals are fully grown, in their first, and probably only, year of reproduction. Both RBCs from fresh blood samples and skeletal MC from tails of preserved lizards were used to examine cell size. We tested the hypothesis that cell size increases with body size at higher latitudes within the range of *A. carolinensis*, as suggested by preliminary data collection from 4 eastern populations (Goodman & Heah 2010) and previous studies in other ectotherms.

MATERIALS AND METHODS

Collection and measurement of specimens and tissues

We collected 29–42 lizards of both sexes from each of 17 populations of *A. carolinensis* throughout southeastern USA (Fig. 1a) in May and Jun of 2006 and 2007. Collection sites included various natural and human-modified habitats but were limited to areas that did not have any artificial sources of water (e.g. sprinklers and irrigation). We were only able to collect 10 and 17 lizards from 2 additional populations in Brownsville, Texas (BV_TX) and southwestern Florida (SW_FL), respectively, due to low population densities in those areas. Attempts were made to collect in more northwestern populations in Texas; however, populations were restricted to urban areas with artificial water sources and/or had such low population densities that they precluded collections during the years of this study (which followed a multi-year drought).

Lizards were measured for mass (with accuracy of 0.01 g) and snout–vent length (SVL, with accuracy of 0.5 cm) within 48 h of collection. In addition, blood samples were taken on a slide after clipping 1 toe, diluted with 0.85% NaCl buffer and covered with a cover slip. Digital images of blood samples were immediately taken under 40× microscopy. Lizards were euthanized via inhalation of isoflourane, fixed in 10% formalin and stored in 95% ethanol.

Numbered grids were added to digital images of RBCs to aid in random selection of cells for measurement. Ten cells from each of 4 images were measured for each lizard using Scion Image software (Scion Corporation, Frederick, Maryland, USA). Average cell size was computed as the surface area (μm^2) of 40 cells (hereafter RBC size).

Preserved lizards were taped to a flat piece of plastic to standardize body position and radiographed laterally in an HP Faxitron 43805N machine (Faxitron Bioptics, LLC, Tucson, Arizona). A metal pin was inserted through the cloaca of each individual to serve as a common landmark visible on both the specimens and in the radiographs. X-ray radiographs were taken with 10-sec exposures at 40 kVp using Kodak Biomax XAR film (Eastman Kodak Co., Rochester, New York). Radiographs were converted to digital images using a scanner, and the distance from the metal pin to the top of the 10th caudal vertebra was measured using Scion Image software to find a common morphological landmark for sampling MC. A metal standard was placed in all x-rays in both years to ensure calibration.

Tails were cut from preserved lizards using a razor blade at a point measured minus 1 cm from the top of the 10th caudal vertebra, as determined individually for each lizard based on radiograph measurements. We initially froze on dry ice, then directly mounted (using

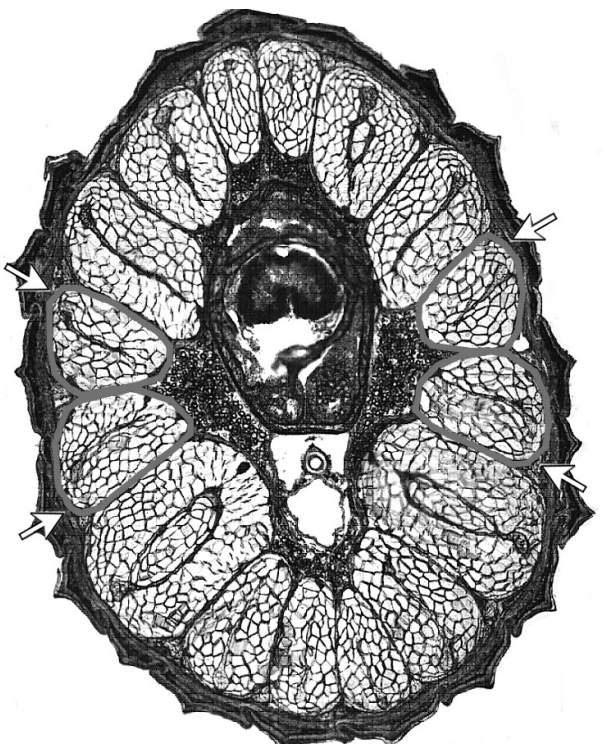

Figure 2 Transverse section through tail of *Anolis carolinensis*, indicating the 4 muscles (circled, as indicated with arrows) sampled in the current study.

cryostat mounting medium) a section of tail with the initial cut face-up. Transverse sections of 50 μm thickness were taken with a cryostat from the initial cut above the 10th post-caudal vertebra. Every other section was floated on 0.1 M phosphate buffer (pH 7.4) and then placed on a chromium aluminum coated slide. While standing in buffer solution, these sections were digitally photographed at 20–80× magnification as needed based on section size. Sections were taken from the anterior to the posterior end of an entire vertebra, as determined by visual examination of distinctive processes in the vertebra. Depending on the size of the lizard, this resulted in 20–40 images. Figure 2 shows a sample of one of these images, with the 4 muscles segments that were sampled circled in red. *Anolis* tails, like those of many lizards, are segmented to facilitate autotomy and muscle segments corresponding to each vertebra interdigitate along the upper length of the tail (including the portion used in this study). The image in which the 4 muscle segments reached their maximum size (just prior to the image in which these muscles expanded and joined with the neighboring muscles) was chosen for data collection. Cross-sectional surface area of the 4 muscles was measured using Scion Image software. All cells within each muscle were counted, and the total area of the muscles was divided by the number of MC (hereafter MC number) to yield an estimate of cross-sectional surface area of MC (μm^2, hereafter MC size).

Climatic data

Climatic data were downloaded from the National Oceanic and Atmospheric Administration's Global Surface Summary of Day database (National Climatic Data Center, Asheville, North Carolina, USA, http://www.ncdc.noaa.gov) for a 20 year period prior to and including the year of lizard collection (1986–2006 and 1987–2007 for populations collected in 2006 and 2007, respectively). This past climate data should represent the selection environment faced by that population over a 20 year historic period. Weather data were usually taken from the weather station closest to a given collecting locality. However, if these data were incomplete, the missing data were obtained from the next nearest station that recorded the data (see Table S1).

For each month within a year, the lowest recorded temperature, total precipitation, and monthly averages of daily mean, maximum, minimum and dewpoint temperatures were calculated. These variables were then used to calculate the following historical estimates: average of lowest recorded temperature and total precipitation in each year (averaged among years); average of mean, maximum, minimum and dewpoint tempera-

Table 1 Eigenvectors for PCtemp and PCprecip demonstrate the correlations between original climate variables and 2 principal components derived from them in the current study

Climate variable	Eigenvector PCtemp	Eigenvector PCprecip
Average of temperature[†]	0.3255	−0.1193
Average of dewpoint temperature[†]	0.3316	0.0141
Average of maximum temperature[†]	0.3256	−0.0629
Average of minimum temperature[†]	0.3186	−0.1563
Average of total precipitation[‡]	0.0373	0.7499
Average of lowest recorded temperature[‡]	0.3290	−0.1087
Average of within-year variance in temperature[†]	−0.3261	−0.0064
Average of within-year variance in dewpoint temperature[†]	−0.3342	−0.0332
Average of within-year variance in maximum temperature[†]	−0.3236	0.0651
Average of within-year variance in minimum temperature[†]	−0.3333	−0.0255
Average of within-year variance in total precipitation[§]	0.1818	0.6140

[†]Annual average and variance of these variables were calculated first by averaging among months within years, then among 20 years prior to collection of study subjects. [‡]For these historical estimates, total precipitation and lowest recorded temperature were calculated for each year, and then averaged over 20 years. [§]For this estimate, within-year variance in total precipitation (among months) was averaged over 20 years.

tures (calculated first among months within years, then among years); average of within-year variance of maximum, minimum and dewpoint temperatures (calculated first among months within years, then among years); and average of within-year variance in total precipitation (from averaging total precipitation among years).

Statistical analyses

Due to high levels of multicollinearity between climatic variables, principal component analysis in JMP 7.0 (SAS Institute, Cary, North Carolina, USA) was used to reduce the 11 climatic variables prior to regression analysis. Principle component analysis (PCA) on the covariance matrix of these variables resulted in 2 principal components explaining 95.6% of variation in the original data. One explained 79.9% of variation (PCtemp) and had weak, positive associations with average maximum, minimum and mean temperature, lowest recorded temperature and dewpoint, and negatively with annual variance in all temperatures (eigenvectors in Table 1 show correlations above 0.30 between these variables and PCtemp). The second principal component (PCprecip) explained 15.7% of variation and was positively associated with average precipitation and variance in precipitation (eigenvectors in Table 1 show correlations above 0.60 between these variables and PCtemp).

Averages within populations (n = 19) were calculated for body size, MC size and number and RBC size. These response variables were then modelled with potential predictor variables of latitude, longitude, PCtemp and PCprecip using PROC REG in SAS 9.1 (SAS Institute). Akaike's information criteria (AIC) was used to determine the best-fitting and most parsimonious model; lower AIC scores reflect maximal variance explained, with penalization for number of explanatory variables included. Use of AIC, as suggested by Burnham and Anderson (2002) for cases with small sample sizes, did not change any findings and, therefore, these figures are not shown. Latitude and PCtemp were the only 2 of 4 explanatory variables with a strong correlation ($r^2 = -0.963$, $P < 0.001$; for all other pairwise correlations $|r^2| < 0.35$, $P > 0.10$). This multicollinearity is not problematic for model selection based on AIC or F-tests of model fit; however, contributions of these 2 variables (partial t-tests of slope and semi-partial [SP] r^2) within a model could not be separated statistically (Stillwell *et al.* 2007).

Within populations, analysis of variance (ANOVA) and regression analysis were used to determine whether there was sexual dimorphism in or effect of SVL on RBC size, MC size and MC number. These analyses were performed in JMP 7.0. Equality of variance and normality were verified for datasets analyzed through ANOVA, and homoscedasticity and normality of error terms were verified for datasets analyzed through regressions.

RESULTS

Variation among populations

Longitude was the only variable in the AIC best-fit model for SVL in *A. carolinensis* based on AIC (Table 2; model: $r^2 = 0.476$, $F = 15.43$, df = 1,17, $P = 0.001$; longitude: slope = 0.462 ± 0.118). Green anoles were larger in the western part of their range (Fig. 1a). Because body size in Florida appeared notably smaller than in the rest of the range, a second analysis was conducted for populations excluding Florida. Exclusion of the 5 Florida populations did not qualitatively alter the simple regression between longitude and SVL ($r^2 = 0.385$, slope = 0.314 ± 0.114, $F = 7.50$, df = 1,12, $P = 0.018$). However, the AIC best-fit model for SVL without Florida populations included only latitude and PCprecip and explained more variation than the previous model for all populations (Table 2; model: $r^2 = 0.803$, $F = 22.44$, df = 2,11, $P < 0.001$). Excluding Florida, body size was negatively related to latitude (SP $r^2 = 0.720$; slope = −1.17 ± 0.18, $t = -6.61$, $P < 0.001$) and tended to be positively related to PCprecip (SP $r^2 = 0.084$; slope = 0.76 ± 0.36, $t = 2.16$, $P = 0.054$) associated with high annual average and variance in precipitation. Adding longitude to this model explained no additional variation after the effects of latitude and PCprecip (Table 2; SP $r^2 = 0.002$; slope = −0.03 ± 0.10, $t = -0.32$, $P = 0.755$), suggesting that body size is not predicted by longitude outside of Florida.

The AIC best-fit model for MC size included latitude and longitude (Table 2; model: $r^2 = 0.457$, $F = 6.72$, df = 2,16, $P = 0.008$). The effect of latitude was marginally non-significant when modelled with the effect of longitude (latitude: SP $r^2 = 0.242$, slope = −38.75 ± 18.57, $t = -2.09$, $P = 0.053$; longitude: SP $r^2 = 0.214$, slope = 22.22 ± 8.85, $t = 2.51$, $P = 0.023$). MC size was larger in western populations, and tended to be larger in southern populations (Fig. 1b). When Florida populations were excluded, the effect of latitude was significant, whereas that of longitude was not (Table 2; model: $r^2 = 0.633$, $F = 9.49$, df = 2,11, $P = 0.004$; latitude: SP $r^2 = 0.630$, slope = −81.70 ± 29.43, $t = -2.78$, $P = 0.018$; longitude: SP $r^2 = 0.003$, slope = 4.10 ± 12.74, $t = 0.32$, $P = 0.753$). The AIC best-fit model for MC size excluding Florida included PCprecip in addition to longitude (Table 2).

Table 2 Results of regression modelling for all possible variables in PROC REG (SAS 9.1), with model r^2 and Akaike's information criteria (AIC) shown

With Florida populations			Without Florida populations		
r^2	AIC	Variables	r^2	AIC	Variables
Snout–vent length					
0.476	46.6	Longitude†	0.720	19.0	Latitude
0.094	57.0	PCprecip	0.707	19.6	PCtemp
0.018	58.5	PCtemp	0.385	30.0	Longitude
0.002	58.8	Latitude	0.022	36.5	PCprecip
0.493	48.0	Longitude, PCtemp	0.803	16.1	Latitude, PCprecip†
0.486	48.3	Latitude, Longitude	0.758	19.0	PCtemp, PCprecip
0.484	48.3	Longitude, PCprecip	0.731	20.4	Longitude, PCtemp
0.523	48.8	Latitude, Longitude, PCtemp	0.805	17.9	Latitude, Longitude, PCprecip
0.501	49.7	Longitude, PCtemp, PCprecip	0.804	18.0	Latitude, PCtemp, PCprecip
0.494	49.9	Latitude, Longitude, PCprecip	0.780	19.6	Longitude, PCtemp, PCprecip
0.525	50.7	Latitude, Longitude, PCtemp, PCprecip	0.805	19.9	Latitude, Longitude, PCtemp, PCprecip
Muscle cell size					
0.309	213.4	Longitude	0.630	150.6	Latitude
0.242	215.1	Latitude	0.597	151.8	PCtemp
0.117	218.0	PCtemp	0.376	157.9	Longitude
0.015	220.1	PCprecip	0.015	164.3	PCprecip
0.457	210.8	Latitude, Longitude†	0.715	148.9	Latitude, PCprecip†
0.427	211.8	Longitude, PCtemp	0.648	151.9	PCtemp, PCprecip
0.312	215.3	Longitude, PCprecip	0.635	152.4	Longitude, PCtemp
0.498	211.3	Latitude, Longitude, PCtemp	0.716	150.9	Latitude, PCtemp, PCprecip
0.495	211.4	Latitude, PCtemp, PCprecip	0.715	150.9	Latitude, Longitude, PCprecip
0.462	212.6	Latitude, Longitude, PCprecip	0.683	152.4	Longitude, PCtemp, PCprecip
0.514	212.7	Latitude, Longitude, PCtemp, PCprecip	0.717	152.8	Latitude, Longitude, PCtemp, PCprecip
Muscle cell number					
0.267	134.9	PCtemp	0.111	87.3	PCtemp
0.262	135.0	Longitude	0.108	87.3	PCprecip
0.180	137.0	Latitude	0.068	87.9	Longitude
0.027	140.2	PCprecip	0.059	88.1	Latitude
0.557	127.3	Latitude, Longitude†	0.379	84.2	PCtemp, PCprecip†
0.527	128.5	Longitude, PCtemp	0.313	85.6	Latitude, PCprecip
0.340	134.9	Latitude, PCtemp	0.226	87.3	Longitude, PCprecip
0.573	128.6	Latitude, Longitude, PCtemp	0.460	84.3	Latitude, Longitude, PCtemp
0.558	129.3	Latitude, Longitude, PCprecip	0.410	85.5	Latitude, PCtemp, PCprecip
0.527	130.5	Longitude, PCtemp, PCprecip	0.383	86.1	Longitude, PCtemp, PCprecip
0.575	130.5	Latitude, Longitude, PCtemp, PCprecip	0.527	84.4	Latitude, Longitude, PCtemp, PCprecip
Red blood cell size					
0.553	79.9	PCtemp	0.149	37.9	Latitude†
0.441	84.1	Latitude	0.134	38.1	PCtemp
0.135	92.4	Longitude	0.041	39.5	PCprecip
0.109	93.0	PCprecip	0.038	39.6	Longitude
0.709	73.7	Latitude, Longitude	0.163	39.6	Latitude, Longitude
0.686	75.2	Longitude, PCtemp	0.150	39.9	Latitude, PCtemp
0.662	76.6	PCtemp, PCprecip	0.149	39.9	Latitude, PCprecip
0.773	71.0	Latitude, Longitude, PCprecip†	0.183	41.3	Latitude, Longitude, PCtemp
0.736	73.8	Longitude, PCtemp, PCprecip	0.163	41.6	Latitude, Longitude, PCprecip
0.709	75.7	Latitude, Longitude, PCtemp	0.150	41.9	Latitude, PCtemp, PCprecip
0.780	72.4	Latitude, Longitude, PCtemp, PCprecip	0.187	43.2	Latitude, Longitude, PCtemp, PCprecip

†The best-fitting and most parsimonious model, as determined by lowest AIC. Only models with the 3 lowest AIC scores are shown for multi-variable models

The AIC best-fit model for MC number also included latitude and longitude (Table 2; model: $r^2 = 0.557$, $F = 10.07$, df = 2,16, $P = 0.002$). Tail muscles contained more skeletal MC in western populations (longitude: SP $r^2 = 0.377$, slope = 3.63 ± 0.98, $t = 3.69$, $P = 0.002$) and more cells in northern populations (latitude: SP $r^2 = 0.180$, slope = 6.73 ± 2.06, $t = 3.27$, $P = 0.005$; Fig. 1c). Exclusion of Florida populations resulted in a lack of correlation between cell number and either latitude or longitude (model: AIC = 89.83, $r^2 = 0.074$, $F = 0.44$, df = 2,11, $P = 0.656$; latitude: SP $r^2 = 0.058$, slope = −0.84 ± 3.14, $t = -0.27$, $P = 0.793$; longitude: SP $r^2 = 0.015$, slope = 0.58 ± 1.36, $t = 0.43$, $P = 0.677$), suggesting that Florida anoles drive trends in MC number. The AIC best-fit model for MC number excluding Florida included PCprecip and PCtemp (Table 2).

The AIC best-fit model for RBC size included latitude, longitude and PCprecip (Table 2; model: $r^2 = 0.773$, $F = 17.03$, df = 3,15, $P < 0.001$). More western populations had larger RBCs (longitude: SP $r^2 = 0.268$, slope = 0.77 ± 0.23, $t = 3.36$, $P = 0.004$), as did more northern populations (latitude: SP $r^2 = 0.441$, slope = 2.87 ± 0.46, $t = 6.23$, $P < 0.001$; Fig. 1d). Populations with larger RBCs tended to have lower values of PCprecip (SP $r^2 = 0.064$, slope = −2.31 ± 1.12, $t = -2.06$, $P = 0.057$), associated with lower values of annual average and variance in precipitation. When the Florida populations were excluded from the analysis, all of these relationships were voided (Table 2; model: $r^2 = 0.163$, $F = 0.65$, df = 3,10, $P = 0.600$; latitude: SP $r^2 = 0.149$, slope = 0.67 ± 0.62, $t = 1.09$, $P = 0.302$; longitude: SP $r^2 = 0.015$; slope = 0.10 ± 0.24, $t = 0.41$, $P = 0.689$; PCprecip: SP $r^2 < 0.001$; slope = −0.01 ± 0.90, $t < -0.01$, $P = 0.997$). Instead, the best-fit model for RBC size excluding Florida included latitude as the only predictor (Table 2).

Variation within populations

In 17 of 19 populations, body size (SVL) was significantly correlated with MC size (Table 3). In SW_FL and BV_TX, the lack of relationship between these variables may have been due to low sample size and limited power. There was no relationship between body size and RBC size or MC number after the Bonferroni method correction for multiple tests. After accounting for the effects of body size, there was no sexual dimorphism in any cell trait (partial F-tests in ANOVA models including SVL, all $P = 0.05$).

DISCUSSION

Climatic variables did not explain the majority of variation in cell size or body size in *A. carolinensis*. A principal component associated with precipitation was selected in only 2 models, although in each case either latitude or longitude was a stronger predictor, and the statistical significance of precipitation was sensitive to inclusion or exclusion of the Florida populations. Overall, latitude and longitude of the 19 populations in this study were the best predictors of cell and body size within the native range of *A. carolinensis*.

In contrast to previous studies, we found that *A. carolinensis* does not follow Bergmann's rule. Michaud and Echternacht (1995) found increasing size with latitude in 8 populations and Goodman (2010) confirmed this trend among 4 populations of *A. carolinensis*; however, these studies were both limited to eastern populations. The current study shows a longitudinal pattern in body size, driven by small body size in Florida populations of *A. carolinensis*. If these are excluded, a latitudinal trend opposing Bergmann's rule becomes evident, with larger anoles in southern, warmer and less seasonal environments. This negative association between body size and latitude mirrors the general pattern in squamates (Ashton & Feldman 2003).

Both historic and current ecological factors may explain the smaller body size in Florida green anoles. Two recent phylogeographic studies of this species delineated 4 to 5 well-supported clades across the native range (Campbell-Station *et al.* 2012; Tollis *et al.* 2012). Florida populations fell into 2 to 3 divergent clades (Tollis *et al.* 2012 did not include locations in Florida that formed 1 clade in Campbell-Station *et al.* 2012), North Carolina populations formed a single clade, and all other populations (forming the majority of the species range) fell into 1 shallowly-diverged clade, indicating a recent expansion in the mid-Pleistocene. The first fossil of *Anolis* in North America comes from the late Miocene, and fossils of *A. carolinensis* in the southeastern USA, including Florida, date to the Pleistocene epoch (Auffenberg 1956; Holman 1995). Tollis *et al.* (2012) date the common ancestor for all clades in this species to approximately 2 million years ago. Particularly during the Pleistocene, peninsular Florida experienced alternate expansion and reduction of area as sea levels rose and fell during glacial and interglacial periods, respectively (Webb 1990). Populations of *A. carolinensis* would have been alternately fragmented and coalesced and, during periods of fragmentation, populations may have been

Table 3 Results for linear regressions of red blood cell (RBC) size and muscle cell (MC) number and size on snout–vent length (SVL) of *Anolis carolinensis* in 19 populations.

Population	*Y*	r^2	Slope ± SE	*t*	df	*P*
E_TN	RBC size	0.02	0.210 ± 0.245	0.86	1, 40	0.396
42, 39	MC number	0.02	0.760 ± 0.918	0.83	1, 37	0.414
	MC size	0.70	94.380 ± 10.102	9.34	1, 37	<0.001*
W_TN	RBC size	0.02	0.260 ± 0.333	0.78	1, 31	0.440
33, 32	MC number	0.00	−0.065 ± 1.375	−0.05	1, 30	0.962
	MC size	0.69	82.423 ± 10.156	8.12	1, 30	<0.001*
NC	RBC size	0.06	0.459 ± 0.322	1.43	1, 30	0.164
32, 32	MC number	0.23	4.028 ± 1.364	2.95	1, 30	0.006
	MC size	0.60	62.129 ± 9.332	6.66	1, 30	<0.001*
SC	RBC size	0.05	−0.291 ± 0.252	−1.15	1, 27	0.259
29, 24	MC number	0.09	−2.905 ± 2.024	−1.44	1, 22	0.165
	MC size	0.69	93.164 ± 13.222	7.05	1, 22	<0.001*
NE_FL	RBC size	0.15	0.599 ± 0.258	2.33	1, 31	0.027
33, 33	MC number	0.00	−0.434 ± 1.278	−0.34	1, 31	0.737
	MC size	0.54	106.798 ± 17.726	6.02	1, 31	<0.001*
NW_FL	RBC size	0.01	0.055 ± 0.287	0.19	1, 31	0.849
33, 29	MC number	0.08	1.508 ± 1.001	1.51	1, 27	0.143
	MC size	0.66	98.082 ± 13.487	7.27	1, 27	<0.001*
M_FL	RBC size	0.10	−0.739 ± 0.340	−1.85	1, 31	0.074
33, 33	MC number	0.03	1.340 ± 1.277	1.05	1, 31	0.302
	MC size	0.53	58.139 ± 9.863	5.89	1, 31	<0.001*
SE_FL	RBC size	0.00	0.029 ± 0.368	0.08	1, 30	0.937
32, 31	MC number	0.07	1.797 ± 1.246	1.44	1, 30	0.160
	MC size	0.60	77.706 ± 11.859	6.55	1, 29	<0.001*
SW_FL	RBC size	0.01	0.352 ± 1.018	0.35	1, 14	0.735
16, 16	MC number	0.02	1.370 ± 2.802	0.49	1, 14	0.632
	MC size	0.21	78.604 ± 41.260	1.91	1, 14	0.078
AL	RBC size	0.00	0.112 ± .322	0.35	1, 32	0.730
34, 30	MC number	0.05	1.698 ± 1.363	1.25	1, 28	0.223
	MC size	0.59	84.320 ± 13.421	6.28	1, 28	<0.001*
MS	RBC size	0.16	0.621 ± 0.260	2.39	1, 30	0.024
32, 31	MC number	0.00	0.163 ± 1.153	0.14	1, 29	0.888
	MC size	0.65	90.518 ± 12.330	7.34	1, 29	<0.001*
GA	RBC size	0.13	0.870 ± 0.400	2.18	1, 33	0.036
35, 34	MC number	0.01	0.591 ± 1.535	0.38	1, 32	0.703
	MC size	0.73	103.369 ± 11.046	9.36	1, 32	<0.001*
AR	RBC size	0.04	0.377 ± 0.315	1.2	1, 38	0.239
40, 38	MC number	0.00	0.110 ± 1.292	0.09	1, 36	0.933
	MC size	0.72	87.188 ± 8.865	9.84	1, 36	<0.001*
N_LA	RBC size	0.05	−0.330 ± 0.238	−1.39	1, 39	0.173
41, 35	MC number	0.18	2.990 ± 1.114	2.68	1, 33	0.011
	MC size	0.63	68.551 ± 9.116	7.52	1, 33	<0.001*
S_LA	RBC size	0.01	0.0993 ± 0.186	0.53	1, 39	0.596
41, 38	MC number	0.10	2.069 ± 1.054	1.96	1, 36	0.057

Table 3 Continued

Population	*Y*	r^2	Slope ± SE	*t*	df	*P*
	MC size	0.79	97.589 ± 8.355	11.68	1, 36	<0.001*
OR_TX	RBC size	0.16	0.598 ± 0.252	2.37	1, 30	0.024
32, 31	MC number	0.16	2.428 ± 1.061	2.34	1, 29	0.026
	MC size	0.72	88.711 ± 10.393	8.54	1, 29	<0.001*
TY_TX	RBC size	0.00	−0.042 ± 0.230	−0.18	1, 37	0.856
39, 37	MC number	0.02	−1.141 ± 1.455	−0.78	1, 35	0.434
	MC size	0.81	95.188 ± 7.711	12.34	1, 35	<0.001*
CC_TX	RBC size	0.10	0.475 ± 0.238	1.99	1, 35	0.054
37, 37	MC number	0.09	1.735 ± 0.920	1.89	1, 35	0.068
	MC size	0.81	102.079 ± 8.378	12.18	1, 35	<0.001*
BV_TX	RBC size	0.16	0.985 ± 0.795	1.24	1, 8	0.251
10, 10	MC number	0.34	4.684 ± 2.030	2.31	1, 8	0.050
	MC size	0.47	51.727 ± 19.330	2.68	1, 8	0.028

Sample sizes for RBC and MC are shown below each population; r^2 and statistics for *t*-tests of slope estimates are shown. **P* denote significance after Bonferroni correction for multiple tests (corrected alpha = 0.00088).

isolated on small islands. Local body size adjustments during these times may be reflected in size patterns observed today.

The smaller body size in Florida anoles may also be attributable to current ecological factors in this area. *A. carolinensis* occupies a unique niche within the southeastern range, and does not have any native competitors that occupy the same or similar microhabitats. However, within the past century, a congeneric competitor, *Anolis sagrei* Duméril and Bibron 1837, has become established on the mainland of Florida. First reported in the USA in the Florida Keys (Garman 1887), mainland populations of *A. sagrei* likely appeared in the 1940s (Oliver 1950; Lee 1985). The species is now widespread in peninsular Florida and is established as far north as South Carolina (Turnbough 2006), and disjunct populations occur from the panhandle of Florida westward into Texas (Dixon 2000). Therefore, the contact time between native mainland populations of *A. carolinensis* and introduced *A. sagrei* ranges from 5 years or less to almost 70 years, equivalent to approximately 5−70 generations, respectively, of *A. carolinensis*. *A. sagrei* occupies a trunk–ground niche which overlaps more closely with the trunk–crown niche of *A. carolinensis* than that of any other native species. Schoener (1970) suggests that size shifts in solitary anole species on islands followed the addition of a second congeneric species. It may be that this is occurring on the mainland of southeastern USA and accounts for the smaller body sizes in Florida relative to the rest of the range. Unfortunately, published data are not available to compare body size estimates for *A. carolinensis* before and after the introduction of *A. sagrei*.

Both MCs and RBCs were larger in western populations. RBC size was related to latitude and, weakly, to precipitation, but these relationships were driven entirely by the Florida populations, which have small RBCs and tend to have high levels of month-to-month variation in precipitation. Outside of Florida, there were no trends in RBC size due to geography or climatic variables. In addition, the inclusion or exclusion of Florida determined whether longitude or latitude was a stronger predictor of MC size, respectively. Number of MCs was positively related to latitude and longitude, respectively. However, these patterns were driven entirely by lower average MC numbers in Florida anoles, associated with small body size, MC size and RBC size in these populations.

Environmental temperatures decrease and seasonality increases with latitude in the southeastern USA, where this study was conducted. Theory suggests that these environments would produce larger cell size for greater metabolic efficiency (Szarski 1983, 1985; Kozlowski *et al.* 2003). However, this prediction was not met in the current study, wherein MC size was negatively related to latitude, and RBC size showed no latitudinal trend outside of Florida. The different patterns in MC and RBC size confirm the importance of examining multi-

ple cell types when studying geographic variation in cell size.

Muscle cell size and body size were positively correlated *within* nearly all populations sampled. Size of MCs may be related to locomotor performance in addition to metabolic efficiency (Arendt & Hoang 2005). Muscle in the tail was chosen for this study because: (i) the structure and orientation of cells allowed comparison of comparable developmental units in lizards of differing body sizes; and (ii) the function of tail muscle at this position was thought to potentially differ less among habitats than, for example, limb muscles, which have been shown to respond developmentally to perch width (Kolbe & Losos 2005). Still, tail muscle is used in locomotion in many lizards, including *A. carolinensis* (Gillis *et al.* 2009), and tail muscle performance needs may relate to body size. Therefore, selection may occur for larger tail MCs along with larger body size within populations.

The current observational study is unable to determine how the interaction between variable environments and gene expression patterns produces the trends in body size and cell size in *A. carolinensis* (Stilwell 2010). Natural selection can act on a population at the level of trait expression and/or the level of trait plasticity. Environmentally-induced changes in growth rates due to the thermal environment can be as large as genetic differences between populations exhibiting different body sizes, as in the lizard *Sceloporus occidentalis* Baird & Girard, 1852 (Sinervo 1990). In *A. carolinensis*, a laboratory experiment demonstrated that egg incubation temperature caused plasticity in RBC size, epithelial cell size and growth rate after hatching (Goodman 2008; Goodman & Heah 2010) (MC size was not included in those studies). Populations differed in plasticity in cell size and plasticity varied between cell types within a population, further complicating any interpretation of geographical patterns in cell size. Additional studies would need to utilize reciprocal transplant experiments across latitudinal and longitudinal gradients to separate the fixed and environmental effects on body and cell sizes.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

Table S1 Locations of collection sites for study of geographic variation in *Anolis carolinensis*, along with locations (and distance from collection sites) of weather stations used for temperature and precipitation data in the current study

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