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ORIGINAL ARTICLE

Temperature-induced plasticity at cellular and organismal levels in the lizard *Anolis carolinensis*

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Abstract

Among ectotherms, individuals raised in cooler temperatures often have larger body size and/or larger cell size. The current study tested whether geographic variation in cell size and plasticity for cell size exist in a terrestrial, ectothermic vertebrate, *Anolis carolinensis* Voigt, 1832. We demonstrated temperature-induced plasticity in erythrocytes and epithelial cells of hatchlings lizards derived from the eggs of females sampled from four populations and incubated at multiple temperatures. Larger cells were produced in hatchlings from cooler treatments; however, hatchling body size was unaffected by temperature. Therefore, temperature-induced plasticity applies at the cellular, but not organismal, level in *A. carolinensis*. In addition, reaction norms for cell size differed among populations. There was a latitudinal trend in cell size and in plasticity of cell size among our study populations. The two southernmost populations showed plasticity in cell size, whereas the two northernmost ones did not. We suggest that selection pressure for larger cell size in northern, cooler environments has restricted plasticity in *A. carolinensis* applied at the cellular level in response to variable incubation environments.

Key words: epithelium erythrocyte, phenotypic plasticity, reptile, temperature.

INTRODUCTION

The temperature size rule (TSR) describes a widespread pattern among ectothermic vertebrates and invertebrates, and its proximate mechanisms and evolutionary explanations have fascinated biologists for decades (Ray 1960; Atkinson 1994). This pattern of slowed growth and developmental rate, but attainment of larger final size in cooler temperatures, has been found in several ecotherms (Atkinson 1994). Higher juvenile growth rates in warmer temperatures may be expected based on the often positive relationship between temperature and activity, locomotion, prey availability, digestion and metabolism (Avery *et al*. 1982; Hertz *et al*. 1983; Stevenson *et al*. 1985; Van Damme *et al*. 1991; Angilletta *et al*. 2002). However, subsequent maturation and punctuation of development at a smaller adult size is unexpected based on the documented benefits of large body size in many ectothermic species (reviewed for reptiles in Goodman 2010). Therefore, the TSR has been called a life history puzzle or paradox (Berrigan & Charnov 1994; Angilletta *et al*. 2004).

Several mechanistic explanations have been proposed for the TSR, although none are universally accepted (Atkinson & Sibly 1997; Angilletta & Dunham 2003; Karl & Fischer 2008). Recently, constraints and/or selective pressures at the cellular level have been proposed to drive TSR patterns at the organismal level (Van der Have & De Jong 1996; Van Voorhies 1996; Atkinson & Sibly 1997). Several authors have developed growth models based on the principle that rates of cellular division and maturation increase faster as temperature rises than does rate of cell

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growth (Van der Have & De Jong 1996; Jarosik *et al*. 2004; Walters & Hassall 2006). Theoretically, the rate of DNA replication is more temperature-dependent than the rate of protein synthesis, because the former depends on the more temperature-sensitive speed of DNA polymerases, whereas the latter depends on the less temperature-sensitive speed of diffusion of smaller molecules. Reaction norms for growth of larger cells in cooler environments have been demonstrated in several lineages of ectothermic invertebrates (reviewed in Arendt 2007). In most but not all cases, larger cell size is associated with growth to larger body size in cooler environments (Van Voorhies 1996; Azevedo *et al*. 2002; Blanckenhorn & Llaurens 2005; Arendt 2007). In contrast to these findings, Atkinson and colleagues (2006) find that temperature-induced plasticity varies among cell types and at different levels of organization. Moreover, several authors have demonstrated that regulation of cell size in insects does not necessarily explain regulation of body size (Nijhout 2003 and references therein).

The tendency for larger-bodied endothermic vertebrates to occur in cooler climates is known as Bergmann's rule (Bergmann 1847; Rensch 1938; Scholander 1955; McNab 1971; Geist 1987; Blackburn *et al*. 1999; Meiri & Dayan 2003). Bergmann originally intended that this pattern apply at the interspecific level, but it is now generally applied at the intraspecific level (Mayr 1956; Ashton *et al*. 2000; Ashton 2002; Meiri & Dayan 2003). Mechanisms driving patterns at interspecific versus intraspecific levels may differ, and so researchers must be careful not to confound the two levels of analysis. Recent studies show that mammals and birds generally follow Bergmann's rule, with larger animals within a species occurring at higher latitudes and lower temperatures (Ashton *et al*. 2000; Ashton 2002; Meiri & Dayan 2003). Several adaptive explanations are proposed for the larger size in endotherms at higher latitudes (reviewed in Cushman *et al*. 1993 and Blackburn *et al*. 1999). Favored explanations include fasting endurance through long winters and minimization of surface area relative to volume for heat conservation (Searcy 1980; Blackburn *et al*. 1999; but see Meiri *et al*. 2005). In ectotherms, and especially in small-bodied species with low thermal inertia (Porter & Gates 1969), heat conservation should not apply as in endothermic species. However, environmental temperature has other implications for ectotherm growth and development (described below). A study by Ashton and Feldman (2003) shows that Bergmann's rule generally applies to turtles, whereas lizards and snakes generally show opposing trends. Adams and colleagues (2008) found that amphibians generally do not follow Bergmann's rule.

Geographic trends in cell size, often in association with a latitudinal or climatic gradient, have been explored on a limited basis and with equivocal results (James *et al*. 1995, 1997; Litzgus *et al*. 2004). Many have speculated on the adaptive value of larger cells in cooler environments, with explanations focused on the lower energetic costs of larger cells standardized per unit area and the difficulty of meeting oxygen demands with larger cells in higher temperatures (Szarski 1983; Woods 1999; Atkinson *et al*. 2006). However, only research with fruit flies (*Drosophila*) has explored the evolution of cell size experimentally, with selection for cold environment survival resulting in increased cell size and body size (Partridge *et al*. 1994).

A hypothesized constraint of temperature-induced plasticity of cell size responsible for the TSR suggests that identical reaction norms must exist across populations and individuals. However, Kingsolver and colleagues (2007) demonstrated that the TSR and related thermal reaction norms can evolve rapidly within a species in natural field conditions. Such rapid trait divergence makes a case against the existence of a general mechanistic constraint as the underlying cause of the TSR.

Among vertebrate ectotherms, the limited work conducted thus far on temperature-induced plasticity of cell size has used aquatic organisms, including tadpoles and several species of fish (reviewed in Arendt & Hoang 2005; Arendt 2007). Aquatic organisms might be expected to respond differently to temperature than terrestrial organisms, because of the potential for oxygen limitation in water and the narrower range of temperatures experienced in comparison to terrestrial habitats (Woods 1999).

So far there have been no attempts to examine temperature-induced plasticity of cell size in a terrestrial, ectothermic species. In addition, to date, only studies with invertebrates have examined how plasticity in cell size might contribute to latitudinal clines in cell and body size. Terrestrial, vertebrate ectotherms may be expected to experience different selection pressures with respect to body size and cell size in comparison to better studied invertebrates and aquatic organisms.

The current study examines whether geographic variation in cell size and plasticity for cell size exist in a terrestrial, ectothermic vertebrate, *Anolis carolinensis* Voigt, 1832 (the Green Anole lizard; Polychrotidae). This is a small, diurnal, arboreal lizard found in 11 states in the southeastern United States (Conant & Collins 1998). Multiple habitat types are occupied by this species throughout its range, which covers approximately 22° longitude and 10° latitude (Minesky 1999). Turnover rates within populations are high each year (estimates of > 90–98%, Gordon 1956; King 1966;

Michael 1972), indicating that few individuals live for more than one reproductive season. In the wild, *A. carolinensis* and other *Anolis* species deposit their eggs on the ground surface, in leaf litter or debris, or in shallow holes dug into the ground (reviewed in Michaud 1990). Therefore, eggs may experience different thermal regimes in different habitats at different latitudes. We tested the null hypothesis that lizards from 5 populations of *A. carolinensis* on a latitudinal cline would show no variation in cell size and no plasticity in cell size in response to differing egg incubation temperatures. Based on the existing published literature on the TSR, we predicted that lizards from northern, cooler populations would have larger cells, and that lizards incubated at cooler temperatures would have larger cells. We predicted that lizards from northern populations would have greater plasticity in cell size, if any variation in plasticity existed, due to the greater range of temperatures experienced in higher latitudes.

MATERIALS AND METHODS

Collection and husbandry of adult study subjects

In May and June 2005, we collected 30–35 adult female lizards from each of four populations in the eastern range of *A. carolinensis*. Most females at this point in the reproductive season had already copulated and, therefore, had stored sperm, which they subsequently used to fertilize eggs while in the laboratory (ovulated and oviposited singly; Licht 1973). Lizards were collected from south of Greenback, Blount County, Tennessee (TN; 35°33.486'N, 84°06.210'W), Augusta, Columbia County, Georgia (GA; 33°32.976'N, 82°02.228'W), Jacksonville, Duval County, Florida (North Florida [NFL]; 30°15.95'N, 81°30.70'W) and east of Orlando, Seminole County, Florida (Middle Florida [MFL]; 28°37.92'N, 81°07.48'W). Lizards were transported to the University of Tennessee, Knoxville and processed within 3 days of capture. Additionally, 65 adult females collected within a 100-km radius of LaPlace, Louisiana (LA; ca. 30°03.93'N, 90°29.18'W) were purchased from a reptile supplier and shipped to the university within 48 h. Upon arrival in the laboratory, mass to the nearest 0.01 g (before toe clipping) of each female was obtained, and snout-vent length (SVL) was measured to the nearest 0.5 mm with a hand-held ruler.

Adult females were housed individually in 3.8 L glass jars with screened lids, a perch, a cover object and Reptisand substrate (ZooMed Laboratories, San Luis Obispo, CA). Enclosures were misted with water twice daily to provide drinking water, and vitamin-dusted crickets were provided every other day. Full spectrum UVB fluorescent lights provided a daily 12:12 h L:D cycle. Temperatures within enclosures were measured with Stowaway Temperature Tidbit Loggers (Onset Computer Corporation, Bourne, MA) and ranged from 25–28 °C daily. Eggs were collected from the sand substrate every other day. After brushing sand off the egg, each was immediately weighed to the nearest 0.01 g and placed in a 345-mL plastic container with 10-g vermiculite and 10-mL water.

Incubation of eggs and collection of hatchlings

Eggs from the TN, NFL and MFL were assigned to incubation treatments of 27 and 30 °C. Eggs from GA were only incubated at 27 °C, because several eggs were used in another experiment. Twice as many females were acquired from LA relative to other populations; therefore, eggs from this population were subject to three treatments: 23.5, 27 and 30 °C. These treatments cover the range of incubation temperatures that produce relatively high survival in *A. carolinensis* (Viets 1993). Eggs were randomly assigned to temperature treatments, and only one egg per treatment was used per female (additional eggs were discarded after all treatments had been covered). Incubation temperatures were recorded every 60 min with temperature loggers (as above). The standard deviation of the 23.5 °C treatment (used for LA only; standard deviation [SD] = 0.86) differed from those of 27 and 30 °C treatments (used

Table 1 Sample sizes for laboratory-reared hatchlings of *Anolis carolinensis* used in analyses of geographic variation and plasticity of cell size

Cell type	Population	23.5 °C		27 °C		30 °C	
		M	F	M	F	M	F
Erythrocytes							
	MFL			7	4	8	6
	NFL			12	14	13	15
	TN			11	13	11	11
	LA	25	23	17	15	18	20
Epithelial cells							
	MFL			5	4	5	3
	NFL			12	13	12	10
	TN			10	12	6	10
	LA	23	24	16	13	17	19

Numbers of males and females (M and F) are indicated for each of three incubation temperatures and each of four populations (LA, Louisiana; MFL, Middle Florida; NFL, North Florida; and TN, Tennessee).

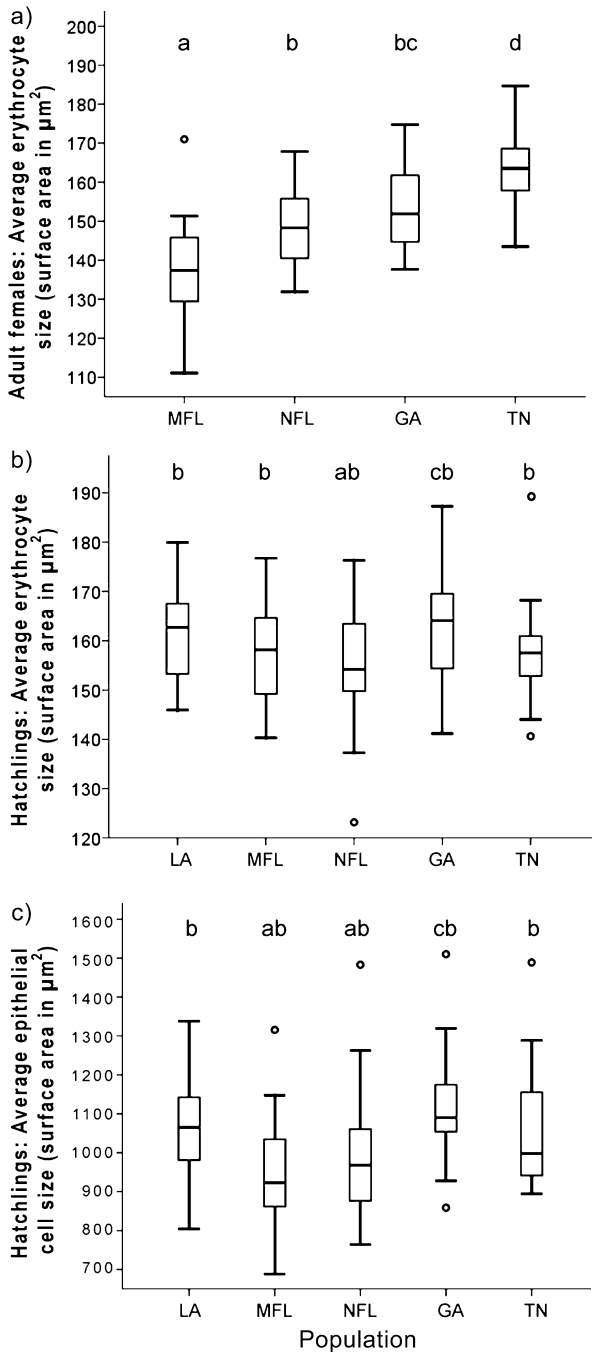

Figure 1 Average cell sizes (surface area in µm²) of (a) erythrocytes of wild, adult female *Anolis carolinensis*, (b) erythrocytes of laboratory-reared offspring from females in five populations (Louisiana, Middle Florida, North Florida, Georgia and Tennesse), and (c) epithelial cells of the laboratory-reared offspring. Boxplots shows the median, interquartile range and outliers for each group. Letters (a) to (d) are in order of increasing means and denote significantly different groups, according to Tukey–Kramer multiple comparison tests.

for all populations; SD = 0.47 and 0.34 °C, respectively) due to mechanical difficulties with one incubator. However, the temperature ranges of all treatments were entirely exclusive of each other.

Positions of egg containers were rotated, and new hatchlings were collected daily (within 24 h of hatching). Mass (before toe clipping or tail clipping) and SVL of hatchlings were recorded. Hatchlings were restrained in the fold of a transparent plastic bag and measured with digital calipers to the nearest 0.5 mm.

Cell collection and measurement

We used blood cells (erythrocytes) and epithelial cells to examine cell size variation and plasticity. Blood samples were collected from adult females in all populations except the LA population within 2 days of capture (via toe clipping; used for identification in additional studies) and from hatchlings in all populations (via tail clipping) within 24 h, of hatching. Epithelial cells were collected only from hatchlings. Within 30 min of opening the incubation container, moist hatchlings dried out and shed an outer layer of epithelial cells. We collected the dorsal, interocular region of this tissue and mounted it on a glass slide in 0.85% NaCl solution. Blood samples were also diluted and mounted on a glass slide using 0.85% NaCl solution. Both cell types were digitally photographed under 400 × power microscopy immediately after collection. Numbered grids were added to digital images of cells, to aid in random selection of cells for measurement. Ten cells from each of four images were measured for each lizard using Scion Image (c) software (Scion Corporation, Frederick, MD). Average cell size was computed as the average of the visible surface area (cross-section) of 40 cells for each cell type.

Statistical analyses

Within wild-caught females and within all hatchlings of each population incubated at 27 °C, linear regression models were used to test for relationships between egg mass, lizard mass or SVL, and average cell size of erythrocytes or epithelial cells. Sizes of erythrocytes were compared among adult females from the four eastern populations using analysis of variance (ANOVA). Sizes of erythrocytes and epithelial cells were compared among laboratory-reared hatchlings using analysis of covariance (ANCOVA) with population and sex as factors, and egg mass as a covariate. All analyses that follow were restricted to one offspring per female per population and treatment.

Potential effects of temperature on cell size were analyzed via ANCOVA within all populations using tempera-

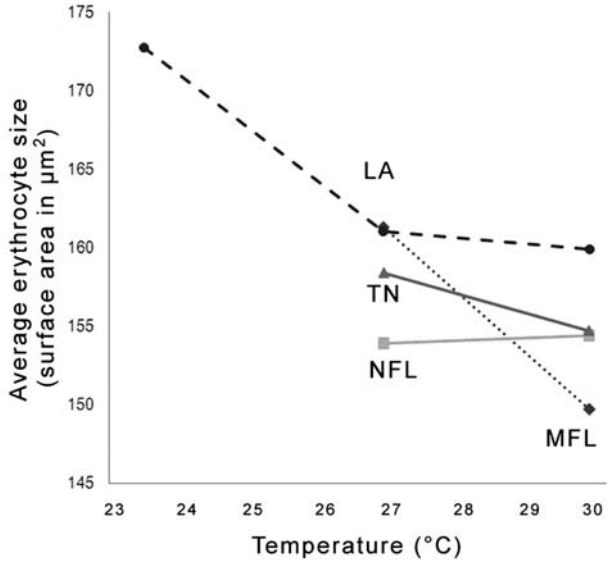

Figure 2 Average erythrocyte sizes (surface area in μm^2) of laboratory-incubated hatchling *Anolis carolinensis* incubated at different temperatures. Hatchlings came from eggs collected from wild-caught females from four populations (Louisiana [◆], Middle Florida [◆], North Florida [■], and Tennessee [▲]). Error bars denote ± 1 standard error.

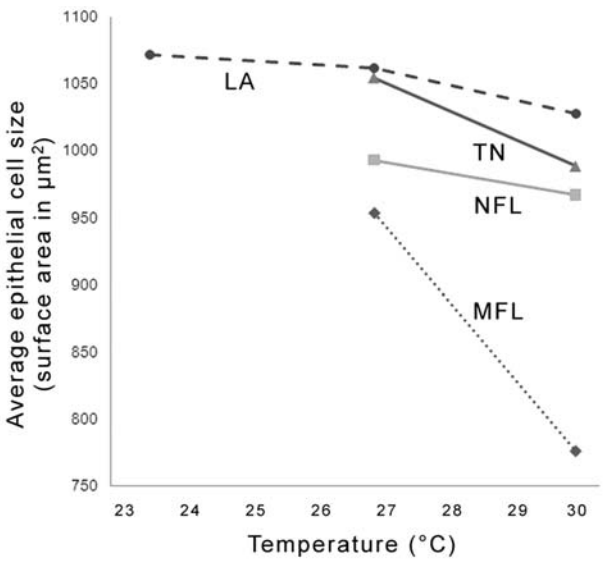

Figure 3 Average epithelial cell sizes (surface area in μm^2) of laboratory-incubated hatchling *Anolis carolinensis* incubated at different temperatures. Hatchlings came from eggs collected from wild-caught females from four populations ((Louisiana [◆], Middle Florida [◆], North Florida [■], and Tennessee [▲]). Error bars denote ± 1 standard error.

ture and sex as factors, and egg mass as a covariate. Interaction effects were included in original analyses; however, they were dropped from the models presented herein because none were statistically significant. Homogeneity of variance among groups was verified for ANOVA and ANCOVA. Following significant results in ANOVA tests, Tukey Kramer multiple comparison tests were used to compare differences among groups. Sample sizes for male and female hatchlings from each incubation temperature and for each population are shown in Table 1. All statistical analyses were performed in NCSS Statistical Software ® (2001) with a critical alpha of 0.05.

RESULTS

Erythrocyte size (average surface area) of wild, adult females differed according to their population of origin, but not body mass (ANOVA, Population: $F_{3,136} = 21.36$, $P < 0.001$; Mass $F_{1,136} = 0.00$, $P = 0.995$). Cell size increased with increasing latitude of populations (Fig. 1a). Within females and within laboratory-reared offspring from each of five populations (eggs incubated at 27 °C only), neither mass nor SVL significantly explained variation in erythrocyte size (Table 2).

At an incubation temperature of 27 °C, geographic variation in erythrocyte size was evident among the five populations (ANCOVA, Population: $F_{4,159} = 2.68$, $P = 0.033$; Egg Mass (covariate): $F_{1,159} = 1.87$, $P = 0.173$; Sex: $F_{1,159} = 0.11$, $P = 0.736$; Fig. 1b). This effect was driven primarily by the inclusion of GA, wherein erythrocytes were larger than those from NFL (Tukey Kramer Multiple Comparison Test (MCT), $P < 0.05$; other populations were intermediate).

Within MFL, erythrocytes from 27 °C were 8% larger in surface area than those from 30 °C (Table 3, Fig. 2). There was no effect of temperature on cell size in NFL (Table 3, Fig. 2) or in TN (Table 3; Fig. 2). In LA, incubation temperature, sex and egg mass all affected erythrocyte size (Table 3). Erythrocytes from 23.5 °C were 7–8% larger in surface area than those from 27 and 30 °C (Tukey Kramer MCT, $P < 0.05$; Fig. 2). Males had erythrocytes that were 3% larger than those of females (average ± SD for males: 168.0 ± 14.5; for females: 162.7 ± 11.6 μm^2).

Within hatchlings from all five populations (eggs incubated at 27 °C only), there were no significant relationships between hatching mass or hatching SVL and epithelial cell size (Table 2). At an incubation temperature of 27 °C, no geographic variation in epithelial cell size was

Table 2 Regression equations for cell size (SA = surface area in μm^2) on mass and snout vent length (SVL) are presented along with sample size (*N*), *t*-value and *P*-value for *t*-tests that slope is equal to zero, and coefficients of determination (R^2)

Wild-caught adult females	Erythrocyte average SA and Mass (g)					Erythrocyte average SA and SVL (cm)				
	Equation	*N*	*t*	*P*	R^2	Equation	*N*	*t*	*P*	R^2
MFL	$y = 151.2 – 7.7$ x	32	–1.188	0.244	0.04	$y = 162.0 – 5.7$ x	32	–0.607	0.549	<0.01
NFL	$y = 157.8 – 4.0$ x	36	–1.220	0.231	0.04	$y = 186.6 – 7.6$ x	36	–1.427	0.163	0.06
GA	$y = 147.3 + 2.1$ x	31	0.649	0.521	0.01	$y = 151.1 + 0.5$ x	31	0.072	0.943	<0.01
TN	$y = 162.6 + 0.1$ x	40	0.061	0.952	<0.01	$y = 146.4 + 3.4$ x	40	0.820	0.417	0.02
	Erythrocyte average SA and Mass (g)					Erythrocyte average SA and SVL (mm)				
	Equation	*N*	*t*	*P*	R^2	Equation	*N*	*t*	*P*	R^2
MFL	$Y = 173.0 – 64.1$ x	15	–0.222	0.828	<0.01	$y = 277.9 – 5.8$ x	15	–0.946	0.362	0.06
NFL	$Y = 147.5 + 29.1$ x	49	0.5325	0.597	<0.01	$y = 171.8 – 0.7$ x	49	–0.372	0.711	<0.01
GA	$Y = 153.6 + 31.7$ x	68	1.2767	0.206	0.02	$y = 141.7 + 0.9$ x	66	0.8166	0.417	0.01

evident among the five populations (ANCOVA,Population: $F_{4,101} = 1.41$, $P = 0.236$; Egg mass (covariate): $F_{1,101} = 0.80$, $P = 0.372$; Sex: $F_{1,101} < 0.01$, $P = 0.961$; Fig. 1c).

In MFL, both temperature and sex affected cell size (Table 3). Cells from 27 °C were 23% larger in surface area than those from 30 °C (Fig. 3). Females had epithelial cells that were 27% larger on average than those of males (average ± SD for males: 775.9 ± 159.9; for females = 981.9 ± 207.8 μm^2). There was no effect of temperature on cell size in any other population (Table 3; Figs 3).

DISCUSSION

The present study is the first to document both temperature-induced plasticity and a latitudinal trend in cell size in a terrestrial, vertebrate species. As predicted, wild female *A. carolinensis* from northern populations had larger erythrocytes than those from southern populations. Although northern females were larger (Goodman 2010), body size did not explain variation in cell size within populations. This result suggests that differences in body size among populations are not responsible for variation in cell size among populations.

The latitudinal trend and magnitude of difference in erythrocyte size among natural populations was not reflected in laboratory-reared offspring from these populations. This discrepancy between mothers and offspring indicates the importance of environmental effects in determining erythrocyte size. Temperature-induced plasticity in erythrocyte size was also supported experimentally in the present study, with erythrocytes from hatchlings incubated at cooler temperatures being larger than those from warmer temperatures in two populations. Contrary to our predictions, epithelial cell size of hatchlings displayed no latitudinal trend in cell size.

Temperature-induced plasticity was found in some populations for the cell types under study. Cooler temperatures produced larger erythrocytes in two of five populations (MFL and LA). Epithelial cells were only affected by egg incubation temperature in one out of five populations in the present study (MFL). These cells were more variable in size than erythrocytes, perhaps as a result of the method of sampling, which only standardized to one area of the body (rather than to a specific scale in that area). Our methods, which were designed to sample nondestructively, might have limited the ability to detect plasticity in epithelial cells.

In all instances of temperature-induced plasticity in the two cell types, colder temperatures resulted in larger cells, following our prediction based on the common pattern described in ectotherms. Variation in plasticity of cell size among populations suggests a flexibility of thermal reaction norms for cell size. Therefore, the thermal sensitivity of cell size should not be thought of as a physiological constraint that might contribute to TSR patterns of body size, contrary to prior speculation (Van der Have & De Jong 1996; Van Voorhies 1996; Atkinson & Sibly 1997). Rather, the current study supports the notion that TSR and related thermal reaction norms might be environmentally dependent and may evolve rapidly under natural conditions (Kingsolver *et al*. 2007; Diamond & Kingsolver 2010).

The southernmost populations in the present study (MFL and LA) demonstrated plasticity in cell size, whereas the northernmost populations (TN and GA) did not. The

Table 3 Results of analysis of variance comparing cell size (average surface area in μm^2) among laboratory-reared hatchlings of *Anolis carolinensis* from different populations, of different sexes, and from eggs incubated at different temperatures

	Erythrocytes				Epithelial			
	Factor/Covariate	df	*F*	*P*	Factor/Covariate	df	*F*	*P*
MFL								
	Temperature	1, 20	5.39	**0.030**	Temperature†	1, 14	4.62	**0.049**
	Sex	1, 20	2.44	0.133	Sex†	1, 14	5.23	**0.038**
	Egg mss	1, 20	0.27	0.612	Egg Mass	1, 13	0.26	0.618
NFL								
	Temperature	1, 49	0.07	0.794	Temperature	1, 42	0.25	0.618
	Sex	1, 49	0.17	0.678	Sex	1, 42	2.50	0.121
	Egg Mass	1, 49	0.31	0.579	Egg mass	1, 42	2.38	0.130
TN								
	Temperature	1, 42	1.68	0.201	Temperature	1, 34	1.95	0.171
	Sex	1, 42	1.43	0.239	Sex	1, 34	0.02	0.893
	Egg mass*	1, 42	3.58	0.065	Egg mass	1, 34	0.58	0.452
LA								
	Temperature	2, 113	17.74	**<0.001**	Temperature	2, 103	1.92	0.151
	Sex	1, 113	5.06	**0.026**	Sex	1, 103	<0.01	0.966
	Egg mass	1, 113	7.64	**0.007**	Egg mass	1, 103	0.64	0.424

Eggs from MFL, NFL, TN were incubated at 27 and 30 °C, while those from LA were incubated at 23.5, 27 and 30 °C.

* Effect was not significant at $p < 0.05$ level after removing other factors/covariates from model.

†Results from reduced model after removing potential covariate of egg mass.

latter might have exhibited plasticity in cells if eggs were exposed to 23.5 °C as they were in the LA population; however, the MFL population exhibited plasticity in cell size even over the more limited treatment difference between 27 and 30 °C. We suggest that selection pressure for larger cell size in northern populations has restricted plasticity of cell size in response to variable incubation environments. The northern sites in the present study have much colder winters, with periods of low feeding activity (Jenssen *et al*. 1996; Bishop & Echternacht 2004), which might exert a selective pressure for uniformly larger and less metabolically expensive cells (Szarski 1983).

Previous studies have found sexual size dimorphism (SSD) in plasticity of cell size in *Drosophila* species, with the larger sex primarily altering cell size in response to rearing temperature and the smaller sex altering both cell size and number (Arendt 2007). We found some SSD in cell size in *A. carolinensis*, but no SSD in plasticity in cell size. Erythrocytes were only slightly larger in males in the LA population, whereas epithelial cells were substantially larger in females in the MFL population. There were no consistent patterns in SSD of cell size in *A. carolinensis*, and the biological significance is therefore unclear. In all wild and laboratory-reared lizards, there were no relationships between body size and cell size. Therefore, any geographic variation or SSD in cell size is probably not a simple extension of body size differences among populations or sexes.

Where plasticity of cell size was demonstrated, cooler temperatures always resulted in larger cells in *A. carolinensis*. These results support a generalized reaction norm of cells growing to be larger in cooler environments, as has been described in many ectotherms (reviewed in Arendt 2007). Despite the plasticity in cell size documented here, a concurrent study demonstrated no temperature-induced plasticity for body size at hatching over the same range of incubation temperatures. However, subsequent growth rates in a common environment differed and were greater in cold-reared juveniles (Goodman 2008). Drawing from these studies, we suggest that the factors shaping cell size on developmental and evolutionary timescales differ from those acting on body size in *A. carolinensis*.

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