8-2009

Geographic Variation and Phenotypic Plasticity of Body Size and Cell Size in the Lizard, Anolis Carolinensis

Rachael Goodman
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Rachael Goodman entitled "Geographic Variation and Phenotypic Plasticity of Body Size and Cell Size in the Lizard, Anolis Carolinensis." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Arthur C. Echternacht, Major Professor

We have read this dissertation and recommend its acceptance:

James A. Fordyce, Jim C. Hall, Daniel S. Simberloff

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a dissertation written by Rachel M. Goodman entitled "Geographic variation and phenotypic plasticity of body size and cell size in the lizard, *Anolis carolinensis*." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Arthur C. Echternacht, Major Professor

We have read this dissertation
and recommend its acceptance:

James A. Fordyce

Jim C. Hall

Daniel S. Simberloff

Accepted for the Council:

Carolyn R. Hodges
Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

GEOGRAPHIC VARIATION AND PHENOTYPIC PLASTICITY OF BODY SIZE AND CELL SIZE IN THE LIZARD, *ANOLIS CAROLINENSIS*

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Rachel M. Goodman
August 2009

ACKNOWLEDGMENTS

First and foremost, I am thankful to Sandy Echternacht, who has been a wonderful graduate advisor, mentor, and friend over the last eight years. In addition to helping and encouraging my research and studies, Sandy has supported teaching and the many other activities that I have pursued as a graduate student and that have shaped my professional interests. I hope to follow in his footsteps and become a mentor who promotes and recognizes success of students relative to their varying talents and goals.

Jim Fordyce, Dan Simberloff, and Jim Hall have provided good input as committee members, and I am grateful for their contributions to this research and the resulting publications. I thank Jim Hall for taking me into his lab and collaborating on the last chapter in this dissertation, and for the many enjoyable hours spent chatting in Walters.

Jessica Welch, Ping Heah, and Doreen Deng were great fun to work with and made important contributions to this research. I also thank Jenna Nolt, Natalie Wyszynski, Amit Jethanandani, Tom Hoskins, and Adam Fuller for helping with experiments and animal care. Justin Walguarnery has been a good buddy and collaborator over the years, helping me enter the *Anolis* world after my Master's work.

Kevin Smith contributed to my research and career development in countless little ways, and kept me laughing all the while. I thank David Etnier for loaning an incubator, and Jim Drake for loaning a camera for use in some experiments. Phyllis Bice, Cheryl Lynn, and Jaime Call have been a great help with keeping things on track, and I will miss stopping into 569 Dabney to catch up with them.

I am grateful for the extensive funding and many resources provided by the Department of Ecology & Evolutionary Biology at the University of Tennessee, Knoxville during my Master's and Doctoral studies. I thank the University for supporting me with the Yates Dissertation Fellowship during the last year, and the Division of Biology for a Science Alliance Award for Outstanding Scholarly Achievement. I also thank the American Museum of Natural History for the Theodore Roosevelt Memorial Fund and the American Society of Ichthyologists and Herpetologists for the Gaige Fund Award that supported my research.

My parents got me started on the academic path a long time ago, and I thank them for always encouraging me to have strong senses of curiosity, adventure, and independence from my youth onward. All of my friends and family made the journey from student to faculty a more enjoyable one. Special thanks go out to my climbing crew (Amy, Dave, Lara, Biff, Adam, and Patrick) for helping maintain sanity in the last two years of research that lacked field work, and to Casey, Tricky, Talky, and the recent addition to the writing support crew, Diane and Gracie. Last and most important, my husband Adam has been a terrific companion and source of balance and levity throughout my graduate work. I thank him for always being my best friend and supporter.

ABSTRACT

Patterns of geographic variation in body size and cell size have long fascinated biologists, and climatic variables have often been considered to explain such patterns. Environmental temperature can profoundly influence the phenotype, including body size. of ectotherms, and reptiles in particular. This dissertation presents four studies that examine how temperature shapes morphology on developmental and evolutionary timescales in the green anole lizard, *Anolis carolinensis*. The first three studies examined variation in and phenotypic plasticity of cell size and body size through laboratory experiments using eggs and juveniles from wild-caught females in five populations of *A. carolinensis*. The fourth study examined geographic variation in body size and cell size in 19 wild populations across the species range. Temperature-induced plasticity in cell size but not initial hatching size was demonstrated. However, subsequent differences in growth rates among juveniles reared in a common laboratory environment indicated a latent effect of incubation temperature on body size. Sampling of body size and red blood cell size from four eastern populations in the range suggested a latitudinal trend in body size and cell size. Rearing of offspring in a common environment indicated differences among populations in juvenile and, potentially, embryonic growth rates contributing to divergence in adult body size. Extended sampling of body size and cell size from 19 populations throughout the range, however, showed that inclusion of Florida populations heavily skewed geographic patterns because of the smaller body and cell size of anoles in the peninsular state. Exclusion of these populations revealed a negative relationship between latitude and both body size and muscle cell size, and no geographic trends in red blood cell size.

TABLE OF CONTENTS

LIST OF TABLES

Part I. Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis* based on experimental manipulations of egg size

Part II. Latent effects of egg incubation temperature on growth in the lizard *Anolis carolinensis*

LIST OF FIGURES

Part I. Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis* based on experimental manipulations of egg size

Part II. Latent effects of egg incubation temperature on growth in the lizard *Anolis carolinensis*

INTRODUCTION

Patterns of geographic variation in morphology within a species interest biologists because they may reflect adaptation to environmental factors that vary throughout a species range. Variation in body size has been well-studied, because of the influence of body size on most aspects of life (Peters 1983; Schmidt-Nielsen 1984). Bergmann (1847) noted that larger-bodied endothermic vertebrates occur in cooler climates, a trend that became known as Bergmann's rule and has been hotly debated since (Rensch 1938; Scholander 1955; McNab 1971; Geist 1987; Blackburn et al. 1999; Meiri & Dayan 2003). The rule was originally intended and has been tested at the interspecific level, but was refined and is generally applied at the intraspecific level (Mayr 1956). 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 larger size in endotherms at higher latitudes (reviewed in Cushman et al. 1993; 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). Among reptiles, turtles comply with Bergmann's rule, whereas lizards and snakes generally oppose it (Ashton & Feldman 2003).

Temperature has a major influence on body size on developmental and evolutionary time scales in ectotherms, affecting both growth and developmental rates. The "temperature-size rule" describes a reaction norm of body size with temperature common to many ectotherms, wherein temperature is negatively related to growth rate but positively related to final body size (Ray 1960; Atkinson 1994). As with Bergmann's rule, the explanation for the temperature-growth rule is unclear though several have been proposed (Von Bertalanffy 1960; Van der Have & De Jong 1996; Atkinson & Sibly 1997; Angilletta & Dunham 2003; Walters & Hassall 2006). One suggested explanation is that catabolism has a higher temperature coefficient than anabolism, leading to faster growth but smaller final size at high temperatures (Von Bertalanffy 1960). A more recent explanation is van der Have and de Jong's model (1996), which shows that ectotherms will follow the temperature-size rule if the slope coefficient for developmental rate with temperature is higher than that of growth with temperature. Walters and Hassall (2006) revised this model to show that ectotherms should follow the rule if the temperature threshold for development is higher than that of growth, downplaying the importance of slope. They also suggested that organisms which oppose the rule must experience selection for either a lowered temperature threshold for development or a higher one for growth. Still, the ultimate reasons why different groups of ectotherms should conform to any of the above proximate mechanisms is unsettled, and may differ among taxa (Angilletta & Dunham 2003).

Ray (1960) and Atkinson (1994) suggested that the temperature-size rule, in association with generally cooler temperatures at higher latitudes, may contribute to patterns of larger body size at higher latitudes in ectotherms. However, several examples of countergradient variation in body size in natural populations have been documented, so contributions of the temperatures-size

rule to geographic variation cannot be taken for granted (Schultz et al. 1996; Yamahira & Conover 2002). Moreover, for taxa such as squamates, which generally run counter to Bergmann's rule, the temperatures-size rule would oppose geographic patterns of body size and therefore cannot account for them.

Incubation temperature is known to have major effects on reptile phenotype, including length of incubation period, size at hatching, growth, thermoregulation, locomotor performance, behavior, morphology, and physiology (reviewed in Birchard 2004). Colder incubation temperatures result in longer incubation periods in most lizards (Shine et al. 1997; Qualls & Shine 1998; Downes & Shine 1999; Ji & Brana 1999; Flatt et al. 2001). Generally, incubation period increases 2-3 times for every 10 ºC of temperature change, although the effect is smaller with increasing temperature (Birchard 2004). Warmer incubation typically results in smaller hatchling lizards (Phillips et al. 1990; Van Damme et al. 1992; Shine et al. 1997; Birchard 2004), in accord with the temperature-size rule. Body proportions including relative tail and limb lengths respond to incubation temperature (Van Damme et al. 1992; Shine et al. 1997; Qualls & Shine 1998; Ji & Brana 1999; Flatt et al. 2001; Du & Ji 2006). Post-hatching growth rates are often affected, though the direction of the temperature effect varies by species (Van Damme et al. 1992; Alberts et al. 1997; Deeming 2004).

Environmental temperature affects growth of reptiles at all life stages but may play the largest role in determination of phenotype at the incubation stage for oviparous species (Birchard 2004) such as the subject of this dissertation, the lizard *Anolis carolinensis* (Polychrotidae). Eggs receive little parental care in most lizards, including *A. carolinensis*, and are therefore subject to temperature fluctuations in their immediate environment (Shine et al. 1997). In contrast, adult

lizards thermoregulate, often with great precision in their environments, and may maintain similar body temperatures in differing thermal settings (Avery et al. 1982). Environmental temperature varies across the geographic range of *A. carolinensis* and also changes throughout the reproductive season within a site, so that consecutive offspring may experience different environments and selective regimes.

Phenotypic plasticity in cells, specifically a negative relationship between cell size and temperature, has been suggested to partially account for geographic variation in ectothermic body size associated with latitude and environmental temperature (Van Voorhies 1996; Atkinson & Sibly 1997). Experimental studies in vertebrate and invertebrate ectotherms have demonstrated that colder temperatures result in larger animals composed of larger cells (reviewed in Arendt 2007). Studies have also examined how genetically fixed variation in cell size versus cell number from natural and laboratory populations of animals contribute to ecogeographic variation in body size (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 *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).

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 and in Arendt 2007). Aquatic organisms might be

expected to respond differently to temperature than terrestrial organisms, because of the potential for oxygen limitation in warmer waters (Woods 1999). So far there have been no attempts to examine temperature-induced plasticity of cell size in a terrestrial, ectothermic vertebrate. Also, 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 green anole, *Anolis carolinensis*, is a small, diurnal, arboreal lizard found in eleven states in the southeastern United States (see Figure IV-1 for range map and study site locations). Multiple habitat types are occupied by this species throughout its range, which covers approximately 22º longitude and 10º degrees latitude. Turnover rates within populations are high each year (estimates of >90% to 98%, Gordon 1956; King 1966; Michael 1972), indicating that few individuals live for more than one reproductive season.

Anolis carolinensis exhibits a seasonal reproductive cycle; mating occurs in April or May through July or August, with some variation among populations (reviewed in Minesky 1999). Females ovulate one egg at a time, alternating ovaries, every 7-14 days on average in the laboratory (Hamlett 1952; pers. obs.). Typically, one egg is laid at a time. Female *A. carolinensis* can store sperm (Conner & Crews 1980). Therefore, females captured from the wild during the mating season will typically lay several fertile eggs in the laboratory. Eggs range in size from 0.200 - 0.515 g at oviposition and are covered in a thin, flexible shell that allows gas and moisture exchange. 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).

Anolis carolinensis exhibits sexual size dimorphism, which is apparently produced by equal sized hatchlings of both sexes followed by more rapid growth of juvenile males (Michaud 1990). A trend of increasing female body size with latitude was documented by Michaud and Echternacht (1995) for eight populations of *A. carolinensis* in the eastern part of its range. However, this trend may or may not apply throughout the entire geographic range. In eastern populations, Michaud and Echternacht (1995) found larger adult females, larger eggs, and larger hatchlings in northern populations. Northern lizards also produced eggs and offspring that were proportionally larger, relative to maternal body size, than southern lizards.

This dissertation extends sampling of body size, and adds collection of cell size, to the entire range of *A. carolinensis* to determine whether there are latitudinal, and other ecogeographic, trends. Phenotypic plasticity of cell size and body size are also examined, to aid in interpretation of ecogeographic patterns. This dissertation is the first examination of geographic variation in cell size and number in an ectothermic vertebrate tied to experimental investigations of temperature-induced plasticity in cell size. Herein I test the hypothesis that cell size increases with body size in higher latitudes within the range of *A. carolinensis*, as suggested by previous work on eastern populations in this species and studies on invertebrate ectotherms. In Part IV, an observational study documents trends among populations in body size and cell size, which may be the product of fixed and plastic responses of populations to environmental variation across the species range. In parts I-III, experimental studies examine differences between a subset of populations in body size, egg size, cell size, and plastic responses of these traits to developmental temperature.

Literature cited

Alberts AC, Perry AM, Lemm JM, Phillips JA. 1997. Effects of incubation temperature and water potential on growth and thermoregulatory behavior of hatchling Cuban rock iguanas (*Cyclura nubila*). Copeia 1997: 766-776.

Angilletta MJ, Dunham AE. 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. Amer Nat 162: 332-342.

Arendt JD. 2007. Ecological correlates of body size in relation to cell size and cell number: patterns in flies, fish, fruits and foliage. Biol Rev Camb Philos Soc 82: 241-256.

Arendt J, Hoang L. 2005. Effect of food level and rearing temperature on burst speed and muscle composition of Western Spadefoot Toad (*Spea hammondii*). Funct Ecol 19: 982-987.

Ashton KG, Tracy MC, de Queiroz A. 2000. Is Bergmann's rule valid for mammals? Am Nat 156: 390-415.

Ashton KG. 2002. Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. Global Ecol Biogeogr 11: 505-523.

Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. Evolution 57: 1151-1163.

Atkinson D. 1994. Temperature and organism size- a biological law for ectotherms? Adv Ecol Res 25: 1-58.

Atkinson D, Sibly RM. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends Ecol Evol 12: 235-239.

Atkinson D, Morley SA, Hughes, RN. 2006. From cells to colonies: at what levels of body organization does the 'temperature-size rule' apply? Evol Dev 8:202-214.

Avery RA, Bedford JD, Newcombe CP. 1982. The role of thermoregulation in lizard biology: predatory efficiency in a temperate diurnal basker. Behav Ecol Sociobiol 11: 261-267.

Bergmann C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Göttinger Studien 3: 595-708.

Birchard GF. 2004. Effects of incubation temperature. Reptilian Incubation: Environment, Evolution, and Behaviour. D. C. Deeming. UK, Nottingham Univ. Press: 103-123.

Blackburn TM, Gaston KJ, Loder N. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. Divers Distrib 5: 165-174.

Conner J, Crews D. 1980. Sperm transfer and storage in the lizard *Anolis carolinensis*. J Morphol 163: 331–348.

Cushman JH, Lawton JH, Manly BFJ. 1993. Latitudinal patterns in European ant assemblages: variation in species richness and body size. Oecologia 95: 30-37.

Deeming DC. 2004. Post-hatching phenotypic effects of incubation in reptiles. Reptilian Incubation: Environment, Evolution, and Behaviour. Deeming DC, Ed. UK, Nottingham Univ. Press, pp. 229-251.

Downes SJ, Shine R. 1999. Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? Oecologia 120: 9-18.

Du WG, Ji X. 2006. Effects of constant and fluctuating temperatures on egg survival and hatchling traits in the northern grass lizard (*Takydromus septentrionalis*, Lacertidae). J Exp Zool 305A: 47-54.

Flatt T, Shine R, Borges-Landaez PA, Downes SJ. 2001. Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. Biol J Linn Soc 74: 339-350.

Geist V. 1987. Bergmann's rule is invalid. Can J Zool 65: 1035-1038.

Gordon RE. 1956. The biology and biodemography of *Anolis carolinensis carolinensis* Voigt. Unpubl Ph.D. Diss. Tulane Univ., New Orleans, Louisiana. Hamlett GWD. 1952. Notes on breeding and reproduction in the lizard *Anolis carolinensis*. Copeia 1952: 183-185.

James AC, Azevedo RBR, Partridge L. 1995. Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. Genetics 140: 659-666.

James AC, Azevedo RBR, Partridge L. 1997. Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. Genetics 146: 881-890.

Ji X, Braña F. 1999. The influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). Comp Biochem Physiol A 124: 205-213.

King FW. 1966. Competition between two south Florida lizards of the genus *Anolis*. Unpubl Ph.D. Diss. Univ. Miami, Coral Gables, Florida.

Litzgus JD, DuRant SE, Mousseau TA. 2004. Clinal variation in body and cell size in a widely distributed vertebrate ectotherm. Oecologia 140: 551-558.

Meiri S, Dayan T. 2003. On the validity of Bergmann's rule. J Biogeogr 30:331-351.

Meiri S, Dayan T, Simberloff D. 2005. Biogeographical patterns in the Western Palearctic: the fasting-endurance hypothesis and the status of Murphy's rule. J Biogeogr 32: 369-375.

McNab BK.1971. On the ecological significance of Bergmann's rule. Ecology 52: 845-854.

Michaud EJ. 1990. Geographic variation of life history traits in the lizard *Anolis carolinensis.* Unpubl Ph.D. Diss., Univ. Tennessee, Knoxville.

Michaud EJ, Echternacht AC. 1995. Geographic variation in the life history of the lizard *Anolis carolinensis* and support for the pelvic constraint model. J Herpetol 29: 86-97.

Michael ED. 1972. Growth rates in *Anolis carolinensis*. Copeia 1972: 575-577.

Minesky JJ. 1999. Development and application of a genetic alogrithm-informational modeling approach to exploratory statistical modeling of lizard-habitat relationships. Unpubl Ph.D. Diss, Univ. Tennessee, Knoxville.

Partridge L, Barrie B, Fowler K, French V. 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. Evolution 48: 1269-1276.

Peters RH. 1983. The Ecological Implications of Body Size. Cambridge, Cambridge Univ. Press.

Phillips JA, Garel A, Packard GC, Packard MJ. 1990. Influence of moisture and temperature on eggs and embryos of green iguanas (*Iguana iguana*). Herpetologica 46: 238-245.

Porter WP, Gates DM. 1969. Thermodynamic equilibria of animals with environment. Ecol Monog 39: 227-244.

Qualls FJ, Shine R. 1998. Geographic variation in lizard phenotypes: importance of the incubation environment. Biol J Linn Soc 64: 477-491.

Ray C. 1960. The application of Bergmann's and Allen's Rules to the poikilotherms. J Morphol 106: 85-108.

Rensch B. 1938. Some problems of geographical variation and species formation. P Linn Soc Lon 150: 275-285.

Schmidt-Nielsen K. 1984. Scaling: Why Is Animal Size So Important? Cambridge, Cambridge Univ. Press.

Scholander PF. 1955. Evolution of climatic adaptation in homeotherms. Evolution 9: 15-26.

Schultz ET, Reynolds KE, Conover DO. 1996. Countergradient variation in growth among newly hatched *Fundulus heteroclitus*: geographic differences revealed by common-environment experiments. Funct Ecol 10: 366-374.

Searcy WA. 1980. Optimum body sizes at different ambient temperatures: an energetics explanation of Bergmann's rule. J Theor Biol 83: 579-593.

Shine R, Elphick EJ, Harlow PS. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. Ecology 78: 2559-2568.

Szarski H. 1983. Cell size and the concept of wasteful and frugal evolutionary strategies. J Theor Biol 105: 201-209.

Van Damme R, Bauwens D, Brana F, Verheyen RF. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. Herpetologica 48: 220-228.

Van der Have TM, De Jong G. 1996. Adult size in ectotherms: Temperature effects on growth and differentiation. J Theor Biol 183: 329-340.

Van Voorhies WA. 1996. Bergmann size clines: a simple explanation for their occurrence in ectotherms. Evolution 50: 1259-1264.

Von Bertalanffy L. 1960. Principles and theory of growth. Fundamental Aspects of Normal and Malignant Growth. Nowinski WW, Ed. Amsterdam, Elsevier, pp. 137-259.

Walters RJ, Hassall M. 2006. The temperature-size rule in ectotherms: may a general explanation exist after all? Am Nat 167: 510-523.

Woods HA. 1999. Egg-mass size and cell size: Effects of temperature on oxygen distribution. Am Zool 39:244-252.

Yamahira K, Conover DO. 2002. Intra- vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? Ecology 83: 1252-1262.

Part I.

Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis* based on experimental manipulations of egg size

The following part is a slightly modified version of a paper to be published in the journal Population Ecology:

Goodman RM. In press. Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis* based on experimental manipulations of egg size. Population Ecology.

As the sole author of this paper, I selected the topic of study, designed the study, collected and compiled the data, conducted all statistical analyses, and wrote the manuscript.

Abstract

Geographic variation in body size is of special interest because it affects nearly all aspects of an organism's life. I examined whether differences in body size among four populations of the green anole lizard, *Anolis carolinensis*, were attributable to maternal investment in egg size and/or growth rates of embryos and juveniles. Larger body size and larger egg size relative to female size in the northern part of the range have been documented in this species, and suggested to be adaptive responses to more extreme winters. The current study confirmed the trends in adult size and egg size in the north, but rejected the trend of larger egg size relative to body size in the north. To control for differences in maternal investment in egg size among populations, I performed yolk removals on eggs from two northern populations to produce comparably sized eggs relative to one southern population. This manipulation was designed to minimize the confounding effect of maternal investment in yolk, the primary energy reserves for eggs, so that intrinsic differences in embryonic growth due to metabolism could be investigated. I found that differences in juvenile and, potentially, embryonic growth rates existed among populations of *A. carolinensis*, both due to and independent of differences in egg size. Juveniles from the northernmost population were bigger not only due to larger egg size, but also due to faster juvenile growth and possibly difference in developmental stage of oviposition or conversion of egg mass to hatchling mass. Larger body size may hold a number of advantages in northern populations of this species, including starvation resistance through winters and better competitive access to food resources and warmer microhabitats.

Introduction

Patterns of geographic variation in morphology within a species interest biologists, because they may reflect adaptation to environmental factors that vary throughout the range of a species. Biologists have long been concerned with explaining large-scale patterns of body size in animals, dating back to Bergmann in 1847. He proposed that larger-bodied endothermic vertebrates occur in cooler climates (in German; 1847), a trend that became known as Bergmann's rule and has been hotly debated since (Rensch 1938; Scholander 1955; McNab 1971; Meiri & Dayan 2003). The traditional explanation was that larger body size allowed heat conservation via a reduced surface area to volume ratio. Bergmann trends have been demonstrated in many endothermic taxa; however, selective pressures other than average environmental temperature and heat conservation have been suggested as explanations (reviewed in Blackburn et al. 1999).

In ectotherms, and especially in small-bodied species with low thermal inertia (Porter & Gates 1969), heat conservation should not apply as in endothermic species. Among reptiles, turtles comply with Bergmann's rule, whereas lizards and snakes generally oppose it (Ashton & Feldman 2003). The green anole lizard, *Anolis carolinensis* (Polychrotidae), follows an intraspecific Bergmann trend in part of its range (Michaud & Echternacht 1995), and the explanation for this is unclear. The current study examines how growth and development vary among embryos and juveniles in populations along a latitudinal gradient for which adult body size is known to vary.

Anolis carolinensis occurs throughout the southeastern United States. A similar life history is found throughout the range, with most lizards hatching growing and over-wintering in

the first and reproducing in the second year, and few lizards surviving beyond the second year (Gordon 1956; King 1966; Michaud 1990; Bishop 2000). Michaud and Echternacht (1995) documented a trend of increasing body size and egg size with latitude in the eastern part of the range. Larger body size may confer greater fitness via enhanced starvation resistance in low resource periods (Schultz & Conover 1999), greater thermal inertia in thermoregulation (Porter & Gates 1969; Stevenson 1985), competitive dominance (Stamps 1984), ability to consume larger and more diverse prey (Vitt 2000), less vulnerability to smaller predators (Ferguson & Fox 1984; Vitt 2000), and increased survivorship (Andrews et al. 2000; however see Warner & Shine 2007). Larger body size may be a consequence of increased maternal investment in eggs or initial offspring size, or increased growth rates of juveniles. These growth rates may in turn be caused by increased resource availability or exploitation, activity levels, foraging efficiency, thermoregulation, competitive ability, or inherent differences in metabolic processes.

Research in reptiles has shown that in addition to juvenile or adult growth rates, populations may differ in developmental stage at oviposition, nutritional and hormonal contents of eggs, and/or embryonic conversion of egg mass into hatchling mass which may be affected by environmental factors and intrinsic metabolic factors (Andrews and Mathies 2000; Oufiero & Angilletta 2006). Therefore, it is possible that maternal effects could differ among populations of *A. carolinensis* and contribute to subsequent observed differences in body size of offspring. Egg size is known to vary among populations of *A. carolinensis;* however previous research indicates that non-polar lipids per unit of egg mass (a primary indicator of reserves for embryonic growth) do not differ significantly between populations of wild collected females (Michaud 1990 and see Conclusions).

In a laboratory study, I examined growth and development of embryos and juveniles from populations along a latitudinal gradient, controlling for egg size (or initial resources) both statistically and experimentally though egg yolk removal. I performed yolk removals on eggs from two northern populations to produce comparably sized eggs relative to one southern population. This manipulation was designed to minimize the confounding effect of maternal investment in yolk, the primary energy reserves for eggs, so that intrinsic differences in embryonic growth due to metabolism could be investigated. I tested the null hypothesis that embryonic growth, incubation period, and juvenile growth, would not vary in a common environment among populations of *A. carolinensis* that differ in latitudinal origin and adult body size. I predicted that juveniles from northern populations would exhibit higher intrinsic growth rates to compensate for inhabiting a colder environment with a shorter growing season associated with higher latitude.

Materials and methods

Animal collection and husbandry of adult females

I collected 31-53 adult female *A. carolinensis* from each of four populations in May - June of 2005: south of Greenback, Blount Co., TN (N 35° 33.486', W 84° 06.210': TN), Augusta, Columbia Co., GA (N 33° 32.976', W 82° 02.228': GA), Jacksonville, Duval Co., FL (N 30° 15.952', W 81° 30.697': North Florida- NFL), east of Orlando, Seminole Co., FL (N 28° 37.915', W 81° 07.482': Middle Florida- MFL). Nearly all females carry sperm at this point in the reproductive season, which they store and use to fertilize eggs (ovulated and oviposited singly) in the laboratory (Licht 1973). Females were transported to the University of Tennessee,

Knoxville and processed and housed in a laboratory within 48 h of capture. They were weighed (to 0.01 g) and measured for snout-vent length and total length (SVL and TL; to 0.5 mm). Females were housed in 3.8 L glass jars with screened lids and containing a perch, cover object, and Repti-sand® substrate (ZooMed Laboratories, Inc.). Enclosures were misted with water daily, and vitamin-dusted crickets were provided every other day. Females were kept in temperatures cycling from 25 during scotophase to 28-30 ˚C during photophase, and placed under UVB and broad-spectrum fluorescent lights on a daily 12:12 h light:dark cycle. Females were returned to their exact sites of capture after collection of eggs for this experiment ceased.

Egg collection and manipulation

Eggs were collected from the sand substrate in each enclosure every other day, or between regular egg checks if laid at the surface of the sand. Collected eggs were measured for mass to the nearest 0.05 g. Eggs were manipulated to reduce size and equalize initial resources for embryos in two of four populations through the removal of egg yolk. This technique has been used in recent years with success in several reptiles (Sinervo & McEdward 1988; Sinervo 1990; Sinervo & Huey 1990; Ji et al. 1999; Radder et al. 2004; Oufiero & Angilletta 2006). In the current study, yolk removal, egg puncture (without yolk removal) and no manipulation (control) were conducted on eggs, with treatment among first, second, and third eggs of each female randomly determined. A maximum of three eggs per female were used in the study, though not all females produced three eggs. Sterile 25 M gage syringes were used to remove an average of 0.064 g (range = 0.018 - 0.133 g; SD = 0.026 g), or 18.2 % of total egg yolk. Hatching success in this study was 87.0 % for control eggs, 81.6 % for punctured eggs, and 77.2 % for eggs with yolk removal (n = 54, 38, and 79 before manipulations, respectively). I verified that yolk removal,

but not the act of egg puncture alone, affected hatchling mass and SVL in GA and TN (ANOVA models contained significant treatment effects but not population effects; Tukey Kramer MCT's showed significant differences between yolk removal versus puncture only and control, with no differences between the latter two treatments).

All eggs were incubated at 27 °C in individual, sealed, 345 mL plastic containers started with 10 g vermiculite and 10 mL water, with positions of eggs within incubators rotated daily. Initial mass of each container was recorded, and water was added to maintain this mass every week after the oviposition date for each egg.

Hatchling husbandry and measurement

New hatchlings were collected daily and measured within 24 hr of hatching. Mass was measured to the nearest 0.05 g, and SVL and TL were measured to the nearest 0.05 mm with digital calipers after restraining hatchlings at the bottom of small transparent plastic bags folded over. Two measurements of length were made for each individual (and repeated if they differed noticeably), and the average of these was used in analyses. Hatchlings were housed haphazardly with regard to population in 38 L enclosures holding several perches and cover objects, and each containing three individuals of roughly the same age. Toe clipping of 1-2 toes allowed for identification of individuals. Enclosures were misted at least two times per day and received UVB and broad-spectrum fluorescent illumination on a 12:12 h light:dark cycle. Temperature profiles in enclosures followed a diurnal cycle, with daily highs of 32-34 ºC in light and 28-30 ºC in shade and nightly lows of 23-25 ºC. Lizards were provided fruit flies, pinhead crickets, and fruit baby food *ad libidum*. Positions of enclosures within the laboratory were rotated once per week. I measured mass and SVL of juveniles weekly for eight weeks, as described above.

Growth rates were calculated as grams per week for mass and mm per week for length (Table I-5; all figures and tables for Part I are located in Appendix I). Offspring were released at capture sites of their mothers at the completion of the experiment.

Statistical analysis of adult female size and egg size

To test the reported trend of adult body size, I compared SVL and mass of adult females among populations using analysis of variance (ANOVA). Post hoc comparisons were then conducted with multiple comparison t tests with Bonferonni corrections of p-values. Egg size before yolk manipulation was compared among populations using analysis of covariance (ANCOVA) with population as the factor, and maternal mass as the covariate (n = 47, 40, 24, and 11 for TN, GA, NFL, and MFL, respectively). Throughout this and the following analyses, all factors and interactions were included in the original model, and any non-significant terms were dropped from subsequent models. Test statistics for non-significant terms from original models are presented in tables, along with test statistics for all significant factors and interactions in reduced models. Post hoc comparisons following all significant ANCOVAs were conducted as t tests on estimated marginal means with Bonferonni corrections of p-values for multiple comparisons. Within each population, egg mass (average mass of the first 3 eggs per female) was regressed against maternal mass using linear regression.

Comparison of egg size for TN and GA eggs subject to yolk manipulations (TN(R) and GA(R), respectively: (R) denotes yolk removal) and unmanipulated eggs from NFL was conducted using ANCOVA with maternal mass as the covariate (sample sizes of 24, 16, and 24 for TN(R), GA(R), and NFL, respectively). I used these three treatments for comparison because egg yolk removals in northern populations aimed at the size of MFL eggs might have caused

excessive mortality, and therefore northern eggs were only reduced to NFL egg size.

Statistical analysis of development and growth of offspring

Incubation periods of unmanipulated eggs from the four populations and from eggs from T(R), GA(R), and NFL were compared in separate ANCOVAs, with egg mass as the covariate. Egg mass conversion (hatchling mass/egg mass), and hatchling mass and SVL were compared among the four populations (unmanipulated eggs) and among TN(R), GA(R), and NFL using ANOVAs or ANCOVAs where appropriate.

I compared growth rates in mass and SVL using Repeated Measures ANCOVAs with population and sex as between subject effects, hatchling mass or SVL (as appropriate) as the covariate, and time (or age of juveniles) as the repeated measure. Full models with all interactions were conducted, and non-significant factors and interactions were removed. Reduced models are presented in Tables I-3 and I-4. Final mass and SVL were compared separately for the four populations (unmanipulated eggs) and for TN(R), GA(R), and NFL using ANCOVAs, with population and sex as factors and hatching mass or SVL as the covariate.

Only one egg per female per treatment was included in all analyses, except where noted otherwise. For all analyses, I verified assumptions of normality of data and homogeneity of variances. All analyses were conducted in SPSS (Release 14.0.0, 2005, SPSS Inc., Chicago, IL) with a critical alpha of 0.05.

Results

Adult female size and egg size

Body size differed among adult females from the four populations (ANOVA: mass – $F_{3, 104} = 23.35$, $P < 0.001$; SVL – $F_{3, 104} = 17.61$, $P < 0.001$), and followed a latitudinal trend with increasing body size in the north (Figs. I-a and I-b). Egg mass differed among populations, and was also influenced by maternal mass (Table I-1; Fig. I-2a). A lack of interaction between female mass and population in the ANCOVA model indicated no geographic variation in the relationship between female body size and egg size (Table I-1). Egg size followed a latitudinal trend, with larger eggs in northern populations (Fig. I-2a). Larger females produced larger eggs (unmanipulated) in all populations; slopes of linear regressions of egg mass against female mass and SVL were significant and positive in each population (Table I-2). After yolk manipulations, eggs from GA(R) were smaller than those from NFL; eggs from TN(R) were of an intermediate size and did not differ from either group (Table I-1; Fig. I-2b).

Embryonic development and growth of offspring

Incubation period of unmanipulated eggs differed due to population of origin, but not due to egg size (Table I-1). Eggs from MFL took longest to incubate, with those from NFL, GA, and TN taking 2.2, 2.6, and 3.2 days less to hatch, respectively (Fig. I-3a). After yolk manipulations, incubation periods did not differ between NFL, GA(R), and TN(R), possibly due to the large variance in the TN(R) group and the exclusion of MFL eggs from this analysis (Table I-1; Fig. I-3b). Egg to hatchling mass conversion (hatchling mass/egg mass) differed among populations (Table I-1). Hatchlings from TN were heaviest relative to their original egg mass compared to those from other populations, though only significantly more so than those from NFL (Fig. I-4a).

Comparison of manipulated eggs from TN(R) and GA(R) and eggs from NFL yielded similar results (Table I-1; Fig. I-4b).

Hatchling mass and SVL differed among populations after adjusting for egg mass (Table I-1; Fig. I-5a). Larger eggs produced heavier and longer hatchlings. Controlled for egg size, hatchling SVL still differed among populations and followed a trend of increasing size with latitude (though no pairwise comparisons with adjusted marginal means were significant with respect to mass). This trend was mirrored in a comparison of manipulated eggs from GA(R) and TN(R) and eggs from NFL. Despite similarly sized eggs after yolk removal, hatchlings from TN(R) were longer and heavier than those from NFL and GA(R) (Table I-1; Fig. I-5b).

Growth rates and final size of hatchlings

Final mass and SVL of juvenile lizards in the four populations (unmanipulated eggs) were affected by age, reflecting overall growth (Tables I-1 and I-3, within subjects effects). Also, interactions were found between age and population, sex, and hatchling mass (but not hatchling SVL; Table I-3, within subjects effects). After eight weeks of growth in a common laboratory environment, juveniles differed in mass and SVL based on their population of origin, sex, and hatching mass or SVL (Table I-1; Table I-3, between subjects effects). Males grew faster than females. Juveniles from TN were heavier (relative to original mass) than those from all other populations (Table I-1; multiple comparison tests with Bonferonni correction; Fig. I-6a). However, at eight weeks of age, juveniles from the four populations did not differ in length when adjusted for sex and hatching length (Table I-1).

Similar results were obtained for the treatments manipulated to attain similar egg sizes (TN(R), GA(R), NFL; Tables I-1 and I-4). Growth rates of mass and SVL differed according to

population and hatchling size (between subject effects in Table I-4; absolute growth rates in Table I-5). After adjusting for hatching mass, TN(R) juveniles were similar in mass to regular TN juveniles, and GA(R) were heavier than regular GA juveniles (Fig. I-6b). Therefore, juveniles from both TN(R) and GA(R) were heavier at the end of the experiment than those from NFL, after adjusting for hatching mass (Table I-1; multiple comparison tests with Bonferonni correction; Fig. I-6b). Juveniles from the TN(R), GA(R), and NFL did not differ in final length, however, when adjusted for sex and hatching length (Table I-1).

Conclusions

This study found differences in juvenile, and potentially, embryonic growth rates among populations of *A. carolinensis*, both due to and independent of differences in the starting point of egg size. Removing yolk from the northern TN and GA produced eggs that were similar in size and slightly smaller than those from the southern NFL population. This manipulation demonstrated that juveniles from the north (in particular, TN) were bigger not only because of larger egg size, but also due to faster juvenile growth and possible difference in developmental stage at oviposition or conversion rate of egg mass to hatchling mass. Convergent evolution of more efficient embryonic growth among northern populations was recently demonstrated in another lizard, *Sceloporus undulatus* (Oufiero & Angilletta 2006). Perhaps eggs in northern populations of *A. carolinensis* have a more efficient developmental process or spend less energy on maintenance in the shorter egg stage, thus explaining the increase in conversion of egg mass to hatchling mass in TN.

Since I did not evaluate embryonic stages of development in freshly oviposited eggs, I could not detect whether larger eggs from the north may have been at advanced developmental stages, possibly accounting for differences in incubation period and egg to hatchling mass conversion. Hormonal and nutritional quality of eggs may have also differed among populations, which could be further examined in future research. However, previous research demonstrated that percentage of nonpolar lipids per unit of egg mass did not differ significantly between wild collected females from a northern and a southern population of *A. carolinensis* (although movement to alternate environments affected lipid quantity; Michaud 1990). Also, lipid mass and egg mass were positively correlated within the two populations in that study, so egg mass was considered to be a reliable indicator of lipid quantity.

This study confirmed the previously described latitudinal gradient in female body size and egg size in *Anolis carolinensis*. However, I did not find a latitudinal trend in the relationship between female body size and relative egg size, as reported by Michaud and Echternacht (1995). That study found a significant positive relationship between female size and egg size in three northern populations, but not in five southern ones. The authors suggested that an optimal egg size exists in the south regardless of female body size, whereas larger eggs are advantageous in the north though a potential optimal egg size is constrained by the body size and pelvic aperture width in females. The contradictory results of the current study might be explained by my inclusion of different study populations or possible plasticity in life history traits between years that is currently unknown (Nussey et al. 2007).

Among all populations, TN had significantly larger hatchlings relative to their original egg size, suggesting that embryos more efficiently converted egg resources into hatchling body

length. Eggs from these populations might also be of higher quality per unit mass; however previous research does not necessarily support this suggestion (Michaud 1990). In the current study eggs from northern populations, which were larger on average, took less time to incubate than those from the southern populations; however, there was no effect of egg mass on incubation period. Differences in incubation period have been shown to translate to fitness consequences in the wild in other lizards (Sinervo & Doughty 1996; Warner & Shine 2007). However, the consequences of the roughly three day difference in incubation period between populations in the current study are unknown.

Absolute and size-adjusted growth rates of juveniles in the common laboratory environment varied among individuals from unmanipulated eggs from all populations. Juveniles from TN and GA were heavier at the end of the experiment after adjusting for initial hatching size, indicating a difference in growth in mass independent of maternally-conferred resources. Their greater length relative to other populations was only due to their larger hatching SVL. I attempted to control for starting size or maternally-conferred resources of juveniles in the northern populations (TN and GA) through yolk removal manipulations to produce similarly sized eggs relative to a southern population (NFL). However, despite producing smaller manipulated egg size than the average for NFL eggs, both TN(R) and GA(R) eggs still resulted in larger hatchlings, creating a disparity in the growth experiment thenceforth. Hatchlings from manipulated TN eggs were significantly larger than those from NFL, and they subsequently grew at a greater absolute rate. However, after correcting statistically for starting size, TN still had higher juvenile growth rates in mass than other all populations in the experiment. This increased growth may be attributable to metabolic processes adaptive to the northern environment, in

support of the latitudinal compensation hypothesis. However, because experimental enclosures housed more than one juvenile, I cannot rule out the possibility that larger size resulted in dominance within enclosures that then affected resource access and growth rates.

Sex affected growth rates in the laboratory, but not hatchling mass, SVL, or egg mass conversion. These results are in accord with findings of Michaud (1990) and Gordon (1956) but contradict the reports by Viets (1993) of slight sexual size dimorphism in hatchings, with males being larger. Although the GA population contained the biggest females (though average size was not significantly bigger than TN), juveniles from GA had slower growth rates in the laboratory, lower egg to hatchling mass conversion, and were smaller than TN juveniles at the end of the experiment. How females from the GA population get to as large as those from TN is unclear, because the TN juveniles outgrow them both as embryos and as juveniles. However, growth subsequent to the first two months may not follow the same patterns as above, in which case size at maturity (seven plus months of age) may not be accurately predicted by early juvenile growth. Also, the laboratory environment in this experiment might not have been natural or optimal for the GA population, and more natural conditions may yield different results.

Larger body size may hold a number of advantages in lizards (see Introduction). In *A. carolinensis*, male body size has been shown to be related to home range size, number of resident females, and dominance in male-male interactions (Greenberg & Noble 1944; Jenssen & Nunez 1998). For green anoles in particular, larger body size may be advantageous in the north because it aids overwinter survival (Michaud 1990). Green anoles do not hibernate, but remain active on warm days throughout the winter though eating and growing little (Jenssen et al. 1996; Bishop & Echternacht 2004). Lipids in fat bodies are used primarily for maintenance energy during the

winter in *A. carolinensis*, in contrast to many temperate lizards whose lipids are used for reproduction (Dessauer 1955; Greenberg & Gist 1985). In other organisms, this potential explanation for Bergmann trends in body size has been supported and termed the "starvation resistance" hypothesis (Brown & Brown 1998; Schultz & Conover 1999; Arnett & Gotelli 2003). A starvation study of fish demonstrated that strong size-dependent winter mortality in a northern (but not southern) population was due to proportionally greater energy depletion in small relative to large fish (Schultz & Conover 1997, 1999). Another adaptive suggestion for larger anoles in the north is that larger juveniles may be better competitors if resources are limited, including warmer, non-freezing overwintering sites that are a subset of the available microhabitats in northern populations (Bishop & Echternacht 2004). Future research should examine overwinter survival of juvenile *A. carolinensis* in northern and southern populations in relation to body size, and determine if any relationship is attributable to starvation resistance through lipid stores or competition for food resources or overwintering sites.

Literature cited

Andrews RM, Mathies T. 2000. Natural history of reptilian development: physiological constraints on the evolution of viviparity. Bioscience 50:227-238.

Andrews RM, Mathies T, Warner DA. 2000. Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. Herpetol Monogr 2000:420-431.

Arnett AE, Gotelli NJ. 2003. Bergmann's rule in larval ant lions: testing the starvation resistance hypothesis. Ecol Entomol 28:645-650.

Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. Evolution 57:1151-1163.

Bergmann C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Göttinger Studien 3:595-708

Bishop DC. 2000. Aspects of the winter behavioral ecology of *Anolis carolinensis* at the northern limit of its range. Unpubl M.S. Thesis. Univ. Tennessee, Knoxville.

Bishop DC, Echternacht AC. 2004. Emergence behavior and movements of winter-aggregated green anoles (*Anolis carolinensis*) and the thermal characteristics of their crevices in Tennessee. Herpetologica 60:168-177.

Blackburn TM, Gaston KJ, Loder. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. Divers Distrib 5:165-174.

Brown CR, Brown MB. 1998. Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. Evolution 52:1461-1475.

Dessauer HC. 1955. Seasonal changes in the gross organ composition of the lizard, *Anolis carolinensis*. J Exp Zool 128:1-12.

Ferguson GW, Fox SF. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. Evolution 38:342-349.

Gordon RE. 1956. The biology and biodemography of *Anolis carolinensis carolinensis* Voigt. Unpubl Ph.D. Diss. Tulane Univ., New Orleans, Louisiana.

Greenberg B, Noble GK. 1944. Social behavior of the American chameleon (*Anolis carolinensis* Voigt). Physiol Zool 17:392-439.

Greenberg DS, Gist DH. 1985. Fat bodies and reproduction in female *Anolis carolinensis*. J Exp Zool 33:277-283.

Jenssen TA, Congdon JD, Fischer RU, Estes R, Kling D, Edmands S, Berna H. 1996. Behavioural, thermal, and metabolic characteristics of a wintering lizard (*Anolis carolinensis*) from South Carolina. Funct Ecol 10:201-209.

Jenssen TA, Nunez SC. 1998. Spatial and breeding relationships of the lizard, *Anolis carolinensis*: evidence of intrasexual selection. Behaviour 135:981-1003.

Ji X, Du WG, Xu WQ. 1999. Experimental manipulation of egg size and hatchling size in the cobra, *Naja naja atra* (Elapidae). Netherlands J Zool 49:167-175.

King FW. 1966. Competition between two south Florida lizards of the genus *Anolis*. Unpubl. Ph.D. Diss. Univ. Miami, Coral Gables, Florida.

Licht P. 1973. Influence of temperature and photoperiod on annual ovarian cycle in lizard *Anolis carolinensis*. Copeia 1973:465-472.

McNab BK. 1971. On the ecological significance of Bergmann's rule. Ecology 52:845-854.

Meiri S, Dayan T. 2003. On the validity of Bergmann's rule. J Biogeogr 30:331-351.

Michaud EJ. 1990. Geographic variation of life history traits in the lizard *Anolis carolinensis*. Unpubl Ph.D. Diss. Univ. Tennessee, Knoxville.

Michaud EJ, Echternacht AC. 1995. Geographic variation in the life history of the lizard *Anolis carolinensis* and support for the pelvic constraint model. J Herpetol 29:86-97.

Nussey DH, Wilson AJ, Brommer JE. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. J Evol Biol 20:831-844.

Oufiero CE, Angilletta MJ. 2006. Convergent evolution of embyronic growth and development in the eastern fence lizard (*Sceloporus undulatus*). Evolution 60:1066-1075.

Porter WP, Gates DM. 1969. Thermodynamic equilibria of animals with environment. Ecol Monogr 39:227-244.

Radder RS, Shanbhag BA, Saidapur SK. 2004. Yolk partitioning in embryos of the lizard, *Calotes vorsicolor*: maximize body size or save energy for later use? J Exp Zool 301A:783-785.

Rensch, B. 1938. Some problems of geographical variation and species formation. Proc Linn Soc Lond 150:275-285.

Scholander PF. 1955. Evolution of climatic adaptation in homeotherms. Evolution 9:15-26.

Schultz ET, Conover DO. 1997. Latitudinal differences in somatic energy storage: adaptive responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). Oecologia 109:516-529.

Schultz ET, Conover DO. 1999. The allometry of energy reserve depletion: test of a mechanism for size-dependent winter mortality. Oecologia 119:474-483.

Sinervo B, McEdward LR. 1988. Developmental consequences of an evolutionary change in egg size: an experimental test. Evolution 42:885-899.

Sinervo B. 1990. The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. Evolution 44:279-294.

Sinervo B, Huey RB. 1990. Allometric engineering: an experimental test of the causes of interpopulational differences in performance. Science 248:1106-1109.

Sinervo B, Doughty P. 1996. Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. Evolution 50:1314-1327.

Stamps JA. 1984. Rank-dependent compromises between growth and predator protection in lizard dominance hierarchies. Anim Behav 32:1101-1107.

Stevenson RD. 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. Amer Nat 125:102-117.

Viets BE. 1993. Lizard reproductive ecology: sex determination and parental investment. Unpubl Ph.D. Diss. Indiana Univ., Bloomington.

Vitt LJ. 2000. Ecological consequences of body size in neonatal and small-bodied lizards in the Neotropics. Herpetol Monogr 14:388-400

Warner DA, Shine R. 2007. Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. Oecologia 154:65-73.

Yamahira K, Conover DO. 2002. Intra- vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? Ecology 83:1252-1262.

Appendix I:

Tables and Figures

Table I-1. Results of ANOVAs and ANCOVAs comparing egg size and characteristics of development and growth in juveniles from four populations (left column: TN, GA, NFL, MFL, unmanipulated eggs) and (right column) from eggs from NFL and from TN and GA eggs subjected to yolk removals (GA(R) and TN(R); see text for details). Non-significant factors and interaction terms were removed from the analyses; reduced models are presented here.

	TN, GA, NFL, MFL			TN(R), GA(R), NFL		
Factor / Covariate	df	F	P	df	F	P
Egg mass						
Pop	3, 117	13.48	<**0.001**	2, 60	9.77	<**0.001**
Maternal Mass	1, 117	105.34	<**0.001**	1, 60	33.30	<**0.001**
Incubation Period						
Pop	3, 119	16.53	<**0.001**	2, 62	1.85	0.165
Egg to hatchling mass conversion (hatchling mass / egg mass)						
Pop	3, 118	7.10	<**0.001**	2, 61	6.65	**0.002**
Hatchling mass						
Pop	3, 117	2.90	**0.038**	2, 60	7.42	**0.001**
Egg Mass	1, 117	56.91	<**0.001**	1, 60	55.87	<**0.001**
Hatchling SVL						
Pop	3, 117	12.13	<**0.001**	2, 60	13.60	<**0.001**
Egg Mass	1, 117	41.97	<**0.001**	1, 60	27.99	<**0.001**
Final Mass						
Pop	3, 116	7.92	<**0.001**	2, 60	11.60	<**0.001**
Sex	1, 116	1.75	0.188	1, 60	13.07	**0.001**
Hatchling Mass	1, 116	31.38	<**0.001**	1, 60	20.59	<**0.001**
Sex*Hatchling Mass	1, 116	5.99	**0.016**	*1, 55*	*0.21*	*0.652*
Final SVL						
Sex	1, 120	21.47	<**0.001**	1, 62	9.83	**0.003**
Hatchling SVL	1, 120	85.37	**0.000**	1, 62	35.57	<**0.001**

Table I-2. Linear regressions of egg size on mass and snout vent length (SVL) of wild adult females from four populations of *Anolis carolinensis* (TN, GA, NFL, MFL; unmanipulated eggs). The coefficient of determination (R^2) and the sample size (n), t test statistic (t) and p-value (P) for t tests of H_o: slope = 0 are shown.

	***x* = Female Mass**					***x* = Female SVL**				
Population	**Equation**	***n***	***t***	***P***	R^2	**Equation**	***n***	***t***	***P***	R^2
MFL	$y = 0.138 + 0.041\ x$	20	2.270	**0.036**	0.223	$y = -0.070 + 0.063\ x$	20	2.295	**0.034**	0.226
NFL	$y = 0.211 + 0.036\ x$	30	3.231	**0.003**	0.272	$y = -0.038 + 0.068\ x$	30	2.935	**0.007**	0.229
GA	$y = 0.159 + 0.063\ x$	25	5.037	**< 0.001**	0.525	$y = -0.151 + 0.099\ x$	25	2.972	**0.007**	0.277
TN	$y = 0.194 + 0.053\ x$	31	5.350	**< 0.001**	0.497	$y = -0.045 + 0.078\ x$	31	3.899	**< 0.001**	0.344

Table I-3. Results of Repeated Measures ANOVAs comparing weekly juvenile growth among *Anolis carolinensis* juveniles from four populations (TN, GA, NFL, MFL; unmanipulated eggs). Age (or time) is the repeated measure.

	Mass (to 8 weeks age)			**SVL (to 8 weeks age)**		
	df	***F***	***P***	***df***	***F***	***P***
Between subjects						
Population	3, 115	13.04	< **0.001**	3, 115	5.60	**0.001**
Sex	1, 115	10.52	**0.002**	1, 115	7.00	**0.009**
Hatch Mass	1, 115	72.98	< **0.001**			
Hatch SVL				1, 115	109.00	< **0.001**
Within subjects						
Age	7, 805	16.91	< **0.001**	7, 805	10.77	< **0.001**
Age x Population	21, 805	4.58	< **0.001**	21, 805	2.17	**0.002**
Age x Sex	7, 805	32.28	< **0.001**	7, 805	23.55	< **0.001**
Age x Hatch Mass	7, 805	8.29	< **0.001**			
Age x Hatch SVL				7, 805	0.98	0.444

* P-values include Greenhouse-Geisser correction for sphericity.

Table I-4. Results of Repeated Measures ANOVAs comparing weekly juvenile growth among Anolis carolinensis juveniles from two northern populations that were products of yolk removal manipulations (TN(R) and GA(R)) and from one southern population (NFL). Age (or time) is the repeated measure.

	Mass (to 8 weeks age)			**SVL (to 8 weeks age)**		
	df	***F***	***P***	***df***	***F***	***P***
Between subjects						
Population	2, 59	20.30	< **0.001**	2, 59	11.79	< **0.001**
Sex	1, 59	3.34	0.073	1, 59	2.70	0.106
Hatch Mass	1, 59	49.17	< **0.001**			
Hatch SVL				1, 59	49.34	< **0.001**
Within subjects						
Age	7, 413	6.26	< **0.001**	7, 413	34.21	< **0.001**
Age x Population	14, 413	7.40	< **0.001**	14, 413	2.84	< **0.001**
Age x Sex	7, 413	12.55	< **0.001**	7, 413	6.65	< **0.001**
Age x Hatch Mass	7, 413	5.63	< **0.001**			
Age x Hatch SVL				7, 413	1.13	0.342

* P-values include Greenhouse-Geisser correction for sphericity.

Table I-5. Absolute growth rates of *Anolis carolinensis* juveniles housed in a common laboratory environment and measured weekly for eight weeks. Mean weekly change in mass and SVL (SE in parentheses) are shown for juveniles from four populations (TN, GA, NFL, MFL; unmanipulated eggs), as well as juveniles from two populations that were products of yolk removal manipulations (TN(R) and GA(R)).

	TN	GA	NFL	MFL
Mass (g/wk)	0.131 (0.007)	0.108 (0.006)	0.093 (0.003)	0.087 (0.006)
SVL (mm/wk)	1.44 (0.06)	1.32 (0.05)	1.34 (0.04)	1.46 (0.08)

	TN(R)	GA(R)
Mass (g/wk)	0.127 (0.006)	0.117 (0.008)
SVL (mm/wk)	1.44 (0.04)	1.44 (0.07)

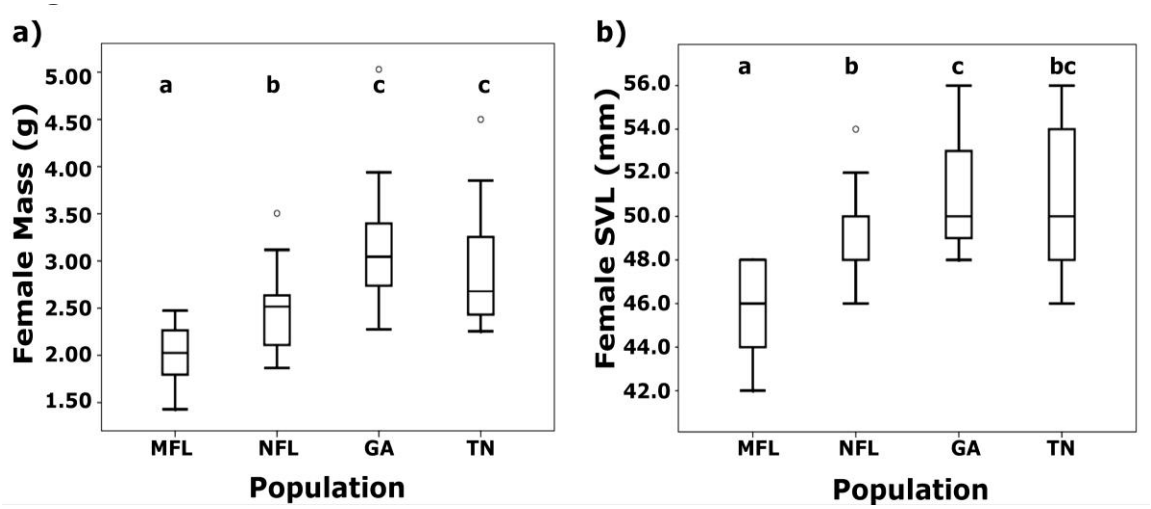

Fig. I-1. (a) Mass and (b) snout vent length (SVL) of adult female *Anolis carolinensis* from four study populations (TN, GA, NFL, MFL; see text for details). Boxplots show the median, interquartile range, and outliers for each population. Letters denote significantly different groups, according to multiple comparison tests with Bonferonni correction.

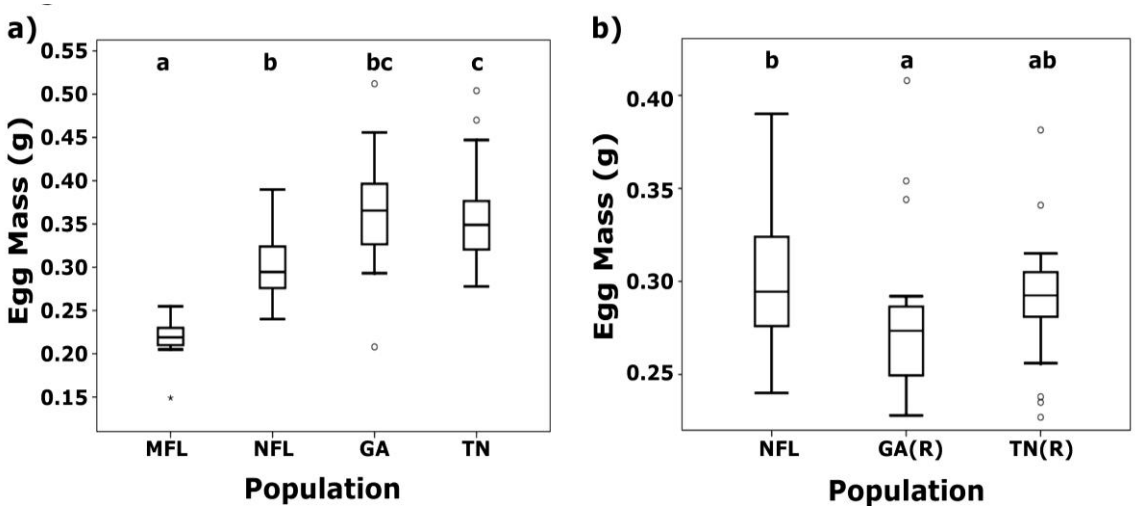

Fig. I-2. Egg masses for (a) unmanipulated eggs originating from four study populations (TN, GA, NFL, MFL; see text for details) and (b) for NFL eggs as well as TN and GA eggs subjected to yolk removals (GA(R) and TN(R)). Boxplots show the median, interquartile range, and outliers for each group. Letters denote significantly different groups, according to multiple comparison tests with Bonferonni correction. Letters a-c are in order of increasing means, which may not match graphical trends because tests are performed on estimated marginal means taking into account covariates (see Table I-1).

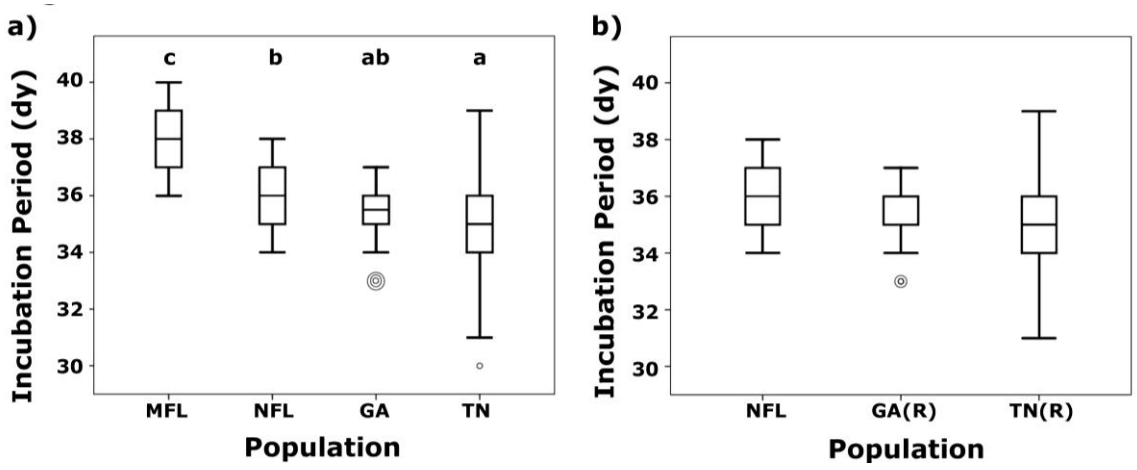

Fig. I-3. Incubation period for hatchlings (a) from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL; see text for details) and (b) from NFL eggs as well as TN and GA eggs subjected to yolk removals (GA(R) and TN(R)). See Fig. I-2 legend for details of boxplot construction. Multiple overlain circles indicate number of outliers at the same value.

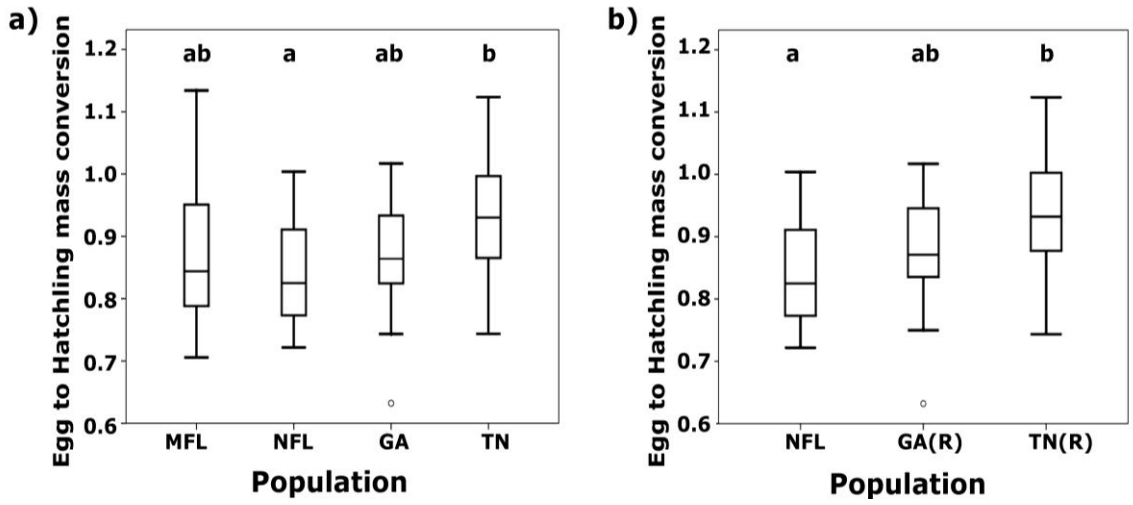

Fig. I-4. Egg mass to hatchling mass conversion (g/g) for hatchlings (a) from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL) and (b) from NFL eggs as well as TN and GA eggs subjected to yolk removals (GA(R) and TN(R)). See Fig. I-2 legend for details of boxplot construction.

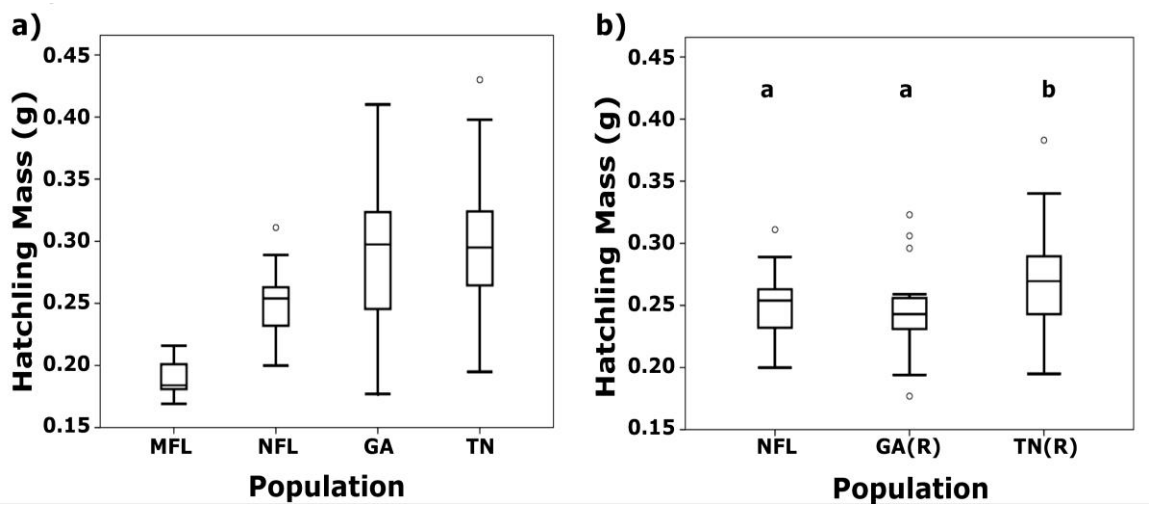

Fig. I-5. Hatchling mass for hatchlings (a) from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL) and (b) from NFL eggs as well as TN and GA eggs subjected to yolk removals (GA(R) and TN(R)). See Fig. I-2 legend for details of boxplot construction.

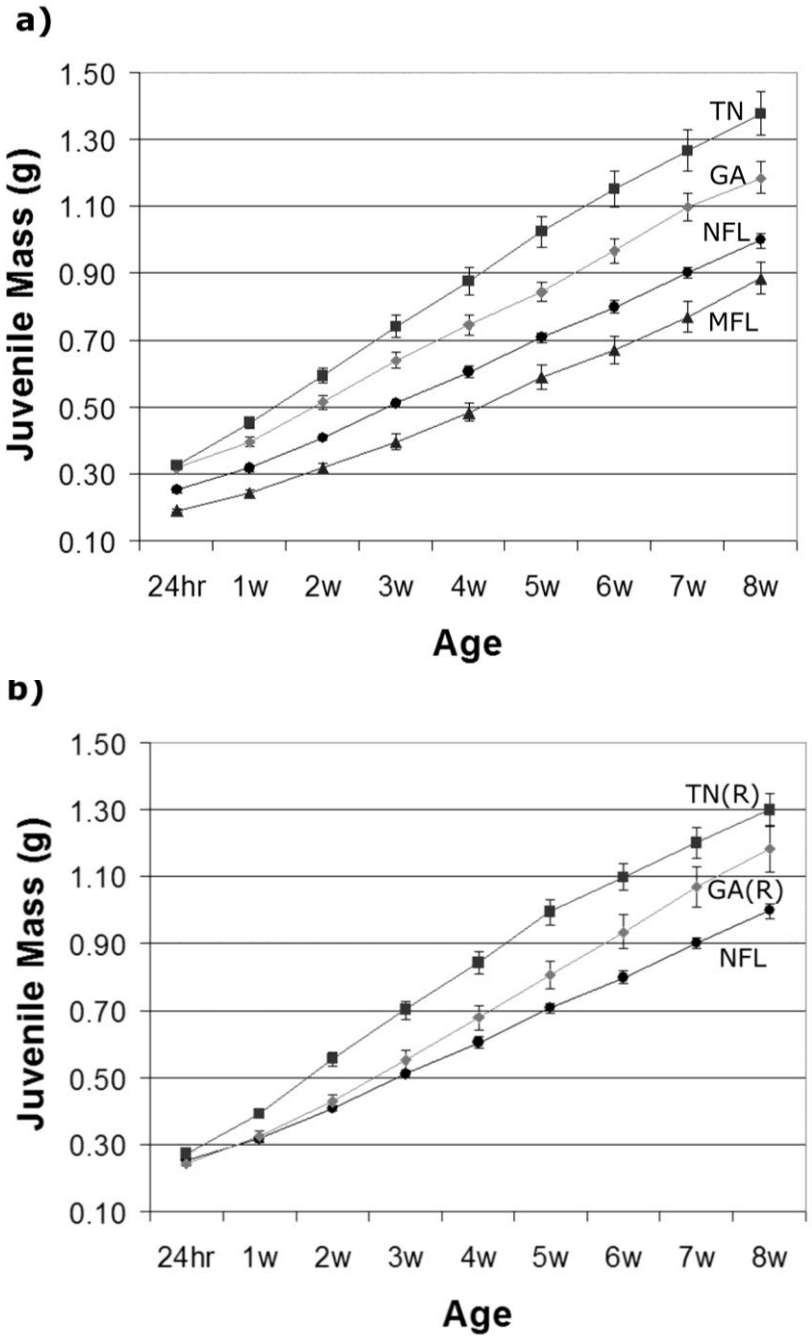

Fig. I-6. Growth in mass of juvenile *Anolis carolinensis* in a common laboratory environment over eight weeks. Juveniles from unmanipulated eggs originating from four populations (TN, GA, NFL, MFL; see text for details) are included in (a). Juveniles from unmanipulated eggs from NFL and from TN and GA eggs subjected to yolk removals (GA(R) and TN(R)) are included in (b). Error bars are ± 1 SE.

Part II.

Latent effects of egg incubation temperature on growth in the lizard

Anolis carolinensis

The following part is a slightly modified version of the published paper:

Goodman RM. 2008. Latent effects of egg incubation temperature on growth in the lizard *Anolis carolinensis*. Journal of Experimental Zoology 309A:1–9.

As the sole author of this paper, I selected the topic of study, designed the study, collected and compiled the data, conducted all statistical analyses, and wrote the manuscript.

Abstract

Varied egg incubation temperatures can result in immediate effects on the phenotype of reptiles, and also latent effects which can augment or contradict immediate effects evident at egg hatching. I examined the effects of incubation temperature on embryonic development, hatching morphology and subsequent growth in multiple populations of the lizard *Anolis carolinensis*. Eggs from wild caught females in four populations were incubated at up to three temperatures, 23.5, 27, and 30 °C. Measures of body size were collected immediately after hatching and weekly thereafter while juveniles were maintained in a common laboratory environment for eight weeks. Cooler incubation temperatures resulted in longer incubation periods but did not affect conversion of egg mass to hatchling mass. Incubation temperature did not affect hatchling mass or SVL, but did affect subsequent growth in both mass and SVL which varied by population. Cooler incubation temperatures generally resulted in greater overall growth over eight weeks of housing all juveniles in a common environment. In *A. carolinensis*, egg incubation temperature had latent effects on juvenile growth despite the absence of any detected immediate effects on hatchling phenotype. Therefore, the total impact and evolutionary importance of developmental environment should not be assessed or assumed based solely on the phenotype of reptiles at birth or hatching.

Introduction

Developmental conditions can have profound effects on the morphology and ecological interactions of organisms and the evolutionary trajectories of populations (Qualls & Shine 1998; Pigliucci 2001; West-Eberhard 2003; Fordyce 2006). The temperature of development in many

ectotherms in particular has been demonstrated to affect several aspects of growth, development, and performance (Atkinson 1994; Johnston & Bennett 1996; Spencer et al. 2006). In many oviparous reptiles, incubation temperature has been shown to affect hatchling size and body proportions (Shine et al. 1997; reviewed in Birchard 2004 and Deeming 2004), growth rates (Van Damme et al. 1992; Alberts et al. 1997; Deeming 2004), locomotor performance (Vanhooydonck et al. 2001; Deeming 2004; Blouin-Demers et al. 2004), and behaviors including thermoregulation (Burger 1998; Downes & Shine 1999; Flatt et al. 2001; Deeming 2004). Although reaction norms may differ dramatically between populations (Niewiarowski & Roosenburg 1993; Iraeta et al. 2006), many studies of temperature-induced plasticity in reptiles focus on one population (reviewed in Deeming 2004; however, see O'Steen 1998; Buckley et al. 2007). The current study examined temperature-induced plasticity in development and growth rates in several populations of the lizard *Anolis carolinensis* with similar life histories, but varying thermal environments.

Body size and egg size both increase with latitude in *A. carolinensis*, and the adaptive and mechanistic reasons are currently under study (Michaud & Echternacht 1995; Goodman, In Press). To contribute to this investigation, I examined how incubation temperature affects embryonic and juvenile growth in this species. The egg is an appropriate stage to subject to different temperatures, because variation in thermal environments of eggs must exist both within and among populations. *Anolis carolinensis* occurs throughout the southeastern United States. The mean monthly temperature differential during the time when eggs are incubated (May-August) between the northern and southern populations in this study ranged from 3.0 °C (July) to 6.6 °C (May) over 1995-2004 (Knoxville, TN and Orlando, FL; NOAA). Female *A. carolinensis*

deposit eggs in and under natural or man-made objects, in shallow soils or leaf litter, or leave eggs exposed or in vegetation (Gordon 1960; Michaud 1990; Echternacht, pers. comm.). Embryos within these eggs do not have the capacity to move to optimal temperatures as adults do, and are therefore subject to the thermal environments where they are located. A previous study found that cooler incubation temperatures produced larger hatchlings in this species but did not examine subsequent growth (Viets 1993; however, see Conclusions for criticism of methods in that study).

Varied egg incubation temperatures may result in immediate effects on the phenotype of reptiles, and also latent effects which may augment or contradict immediate effects evident at egg hatching. Based on previous studies in lizards, I predicted that cooler incubation temperatures would produce larger hatchlings relative to original egg size that subsequently grow faster as juveniles than those incubated at warmer temperatures. Specifically, I tested the null hypothesis that juveniles from different incubation temperatures would exhibit similar incubation periods, hatchlings sizes, and growth rates during eight weeks in a common laboratory environment.

While some studies examine plasticity of morphology at the hatchling stage only (examples in Deeming 2004), I chose to examine post-natal growth as well to determine whether initial differences in morphology would persist, be amplified, or be compensated for with time (e.g. Joanen et al. 1987; Elphick & Shine 1998; Ji et al. 2003; Spencer et al. 2006; Buckley et al. 2007). Incubation or developmental temperature affects thermoregulation in juvenile reptiles including *A. carolinensis* (Blouin-Demers et al. 2000; Blumberg et al. 2002; Goodman & Walguarnery 2007), which in turn may affect growth rates. This potential effect was limited in

the current study by rearing juveniles in a common environment with some, but limited, opportunities for thermoregulation.

Materials and methods

Collection and husbandry of adult females

In May and June of 2005, I collected 31-53 adult female *A. carolinensis* from each of three populations: south of Greenback, Blount Co., TN (N 35° 33.486', W 84° 06.210': TN), Jacksonville, Duval Co., FL (N 30° 15.95', W 81° 30.70': North Florida- NFL), and east of Orlando, Seminole Co., FL (N 28° 37.92', W 81° 07.48': Middle Florida- MFL). Additionally, 69 originally wild-caught females were purchased from a reptile supplier in LaPlace, LA (approx. N 30° 03.93', W 90° 29.18': LA) and shipped to Tennessee in June and July of 2005. Females were all measured upon arrival at the laboratory and housed individually as described by Goodman and Walguarnery (2007).

Collection and incubation of eggs

Eggs were collected from the sand substrate in each enclosure at least every other day and immediately measured for mass to the nearest 0.05 g. Eggs were incubated in airtight, 345 mL plastic containers with 10 g vermiculite and 10 mL water at 23.5, 27, or 30 °C. I chose experimental treatments covering a wide range of incubation temperatures known to produce viable hatchlings in the laboratory (Viets 1993). Because of additional experiments on these subjects, eggs from the three eastern populations (MFL, NFL, TN) were subject to two incubation temperatures (27 and 30 °C), while eggs from the LA population were subject to three temperatures (23.5, 27 and 30 °C). The total weight of the egg, water, and vermiculite was

recorded, and water was added to maintain this weight every week after the oviposition date for each egg. Only one egg per treatment per female was allowed, and the order of eggs in all treatments was distributed evenly by random assignment of the first egg for each female (and of the second egg in LA). Incubation temperatures were recorded every 60 min with Stowaway Temperature Tidbit Loggers (Onset Computer Corporation, Bourne, MA). The standard deviation of the 23.5 ˚C treatment (used for LA only; SD = 0.86 ˚C) differed from those of 27 and 30 ˚C treatments (used for all populations; SD = 0.47 and 0.34 ˚C, respectively) due to logistic difficulties with one incubator. However, the temperature ranges of all treatments were entirely exclusive of each other. I rotated positions of eggs within incubators and collected new hatchlings daily.

Husbandry and measurement of offspring

I measured snout vent length (SVL), tail length (TL), and mass of hatchlings within 24 hr of hatching and prior to first feeding. Sex, which is genetically determined in this species, was ascertained by the presence (male) or absence (female) of enlarged post-anal scales, as viewed under a magnifying glass. Hatchlings from the three eastern populations were housed randomly with regard to population and incubation temperature in 38 L glass aquaria holding perches and cover objects and visual barriers between adjacent aquaria. Hatchlings from LA were not included in the growth portion of this study. Each aquarium contained three individuals of roughly the same age, identified by unique combinations of 1-2 clipped toes. I verified that sex, population, and incubation temperature had no influence on the order of introduction into the enclosures. Aquaria were misted at least two times per day, and fruit flies, pinhead crickets, and fruit baby food were provided *ad libidum*. Lights provided UVB and broad-spectrum fluorescent

illumination on a 12:12 hour light:dark cycle. Temperatures followed a diurnal cycle within the aquaria, with daily highs of 32-34 ºC in light and 28-30 ºC in shade and nightly lows of 23-25 ºC. I rotated positions of enclosures within the laboratory once per week, and measured mass and SVL of juveniles weekly for eight weeks. For the eastern populations, females were returned to their exact sites of capture after collection of eggs for this experiment ceased, and offspring were released at capture sites of their mothers at the completion of the experiment.

Statistical analysis

I analyzed the effects of incubation temperature on incubation period, conversion of egg mass to hatchling mass, hatchling mass, SVL, body proportion (TL/SVL), and body condition (mass/SVL) using analyses of covariance (ANCOVAs) with temperature and population as factors, and egg mass as the covariate (samples size of 44, 50, and 25 for TN, NFL, and MFL, respectively; 58 and 61 for 27 and 30 ºC, respectively). Similar analyses excluding the factor of population were conducted for the LA population, wherein eggs were incubated at three temperatures (sample sizes of 50, 32, 37 for 23.5, 27 and 30 ºC, respectively). Sex had no significant effects in the above analyses, and therefore reduced models are presented in Table II-1. I examined growth rates by analyzing the effects of incubation temperature on mass and SVL during eight weeks in the laboratory with repeated measures (RM) ANCOVAs (samples size of 44, 50, and 25 for TN, NFL, and MFL, respectively; 58 and 60 for 27 and 30 ºC, respectively). Temperature, population, sex, and hatchling mass or SVL were between subjects factors, and within subject factors were time and time interactions with temperature, population, sex, and hatching mass or SVL. Because significant effects of incubation temperature on growth and final size were demonstrated, I analyzed the effects of temperature, sex, and hatchling mass on

final mass, and temperature, sex, and hatchling SVL on final SVL, within each of the three eastern populations using ANCOVAs.

I verified assumptions of normality of residuals and homogeneity of variance for ANCOVAs. Additionally, Greenhouse-Geisser corrections are included in p-values for the within subjects effects tests in RM ANCOVAs, because of violations of sphericity as indicated by Mauchly's test. I performed all statistical analyses in SPSS (Release 14.0.0, 2005, SPSS Inc., Chicago, IL) with a critical alpha of 0.05.

Results

Cooler incubation temperatures resulted in longer incubation periods in all populations (Table II-1; Fig. II-1; all figures and tables for Part II are located in Appendix II). However, incubation temperature did not affect conversion of egg mass to hatchling mass in any population (Table II-1). No temperature-induced plasticity was detected in any of the four populations with respect to hatchling mass or SVL after controlling for egg mass (Table II-1). Incubation temperature also had no effect on body proportion (TL/SVL) or condition (mass/SVL) in any of the populations (Table II-1).

During eight weeks of growth in a common laboratory environment, incubation temperature affected growth in mass among hatchlings from the three eastern populations after accounting for effects of sex, population, and hatching mass (between subjects effect, Table II-2; Figs. II-2a-c). Temperature, sex, and hatching mass interacted with time in their effect on growth in mass (within subjects effect; Table II-2). The same results were evident for *length* of hatchlings for all between subjects factors: temperature, population, sex, and hatching SVL

(Table II-2; Figs. II-3a-c). Males generally grew faster than females in terms of mass and length (Table II-2). Within subject effects on growth in SVL were also similar; however, there was no temperature by time interaction effect, suggesting that the effect of incubation temperature remained constant through time (within subjects effect; Table II-2).

By the end of eight weeks in a common environment, incubation temperature had affected final mass and length of juveniles after controlling for egg mass, population, and sex (Table II-2; Figs. II-2 and II-3). Within MFL, the 27 ºC incubation treatment produced greater final mass compared to 30 ºC (Table II-3; Fig. II-2a). However, there was only a non-significant trend for longer hatchlings resulting from 27 ºC as compared to 30 ºC, after adjusting for hatchling SVL (Table II-3; Fig. II-3a). In NFL, cooler incubation produced greater final mass and length of hatchlings, after adjusting for hatchling mass and SVL (Table II-3; Figs. II-2b and II-3b). In TN, the cooler incubation treatment led to greater final mass relative to hatchling mass, but had no effect on final length (Table II-3; Figs, II-2c and II-3c).

Conclusions

Incubation temperature affected the length of incubation period, as predicted for lizards and ectotherms in general. Cooler developmental temperatures typically slow development in ecotherms (Atkinson 1994; Deeming 2004), and this was the case for *A. carolinensis.* Despite this extended embryonic stage and contrary to my prediction, I detected no temperature-induced plasticity in embryonic growth in *Anolis carolinensis* incubated over 23.5 - 30 °C. Conversion of egg mass to hatchling mass shows variation among populations in this species (Goodman, In Press), indicating some evolutionary flexibility if not immediate plasticity for this trait.

Interestingly, Viets (1993), who also used lizards from LaPlace, LA, found that cooler incubation temperatures (21.5 - 25 °C) produced significantly larger hatchlings than warmer temperatures (28 - 32 °C). His results may actually have reflected desiccation of the substrate in warmer temperatures, as drier incubation substrates have been shown to produce smaller hatchlings in *A. carolinensis* (Michaud 1990). Whereas I used airtight containers to incubate eggs, and refilled lost or used moisture weekly, Viets flushed the air in his containers daily and did not add moisture during the incubation period.

Although the temperature treatments used in this study may not be those experienced in nature, they cover the range of constant temperatures under which *A. carolinensis* can be successfully incubated, and are thus well-suited to test the existence of temperature-induced plasticity in this species. Temperature-induced plasticity in hatchling morphology has been found in many other species of lizards using a range of temperatures similar to the current study (Deeming 2004). However, some species appear unaffected within a range of incubation temperatures such as that used to test *Anolis carolinensis* (Deeming 2004; Angilletta et al. 2006). Cooler temperatures produce larger hatchlings in most species of reptiles that exhibit temperature-induced plasticity, and this is usually accompanied by an increase in the length of incubation period (Birchard 2004; Deeming 2004). This pattern is one demonstration of the "temperature-size rule" common to ectotherms, characterized as slower growth and development but greater final size in cooler temperatures (Ray 1960; Atkinson 1994). The current study does not fit this pattern, however, in that 1) embryonic growth in *A. carolinensis* does not appear to be affected by temperature in the range tested, and 2) post-natal growth is greater (in absolute rate) in cool-reared individuals. The possible adaptive explanation for the temperature-size rule is still

debated in the literature (Berrigan & Charnov 1994; Van der Have & De Jong 1996; Angilletta & Dunham 2003); the exceptions to the rule in this study are also of uncertain significance.

As predicted, cool-incubated *A. carolinensis* displayed higher growth rates in the laboratory, in terms of mass in all populations, and body length in one population. Higher growth rates may have been attributable to behavioral advantages in speed or dominance caused by cool-incubation (which have been noted for other species, but were not examined in the current study) that then caused differential access to food in the group housing situation. However, prey of diverse sizes were available *ad libitum* throughout the study. A more likely explanation for differences in growth rate is that the metabolism and physiology of lizards were somehow adjusted in the embryonic stage upon exposure to cooler temperatures. Metabolism, digestion and growth rates are positively related to temperature in reptiles (within limits; Andrews 1982; Sinervo & Adolph 1989; Avery et al. 1993; Wang et al. 2002), and incubation temperature has been shown to affect thermoregulation in *A. carolinensis* from the Louisiana population used in the current study (Goodman & Walguarnery 2007). The upper limit (but not median or lower limits) of selected temperatures was greater in hatchlings from 27 °C than those from 30 °C, although these differences had disappeared by around 23 days of age. Therefore, even if some selection of warmer temperatures within aquaria accounted for increased growth of cool-incubated individuals in the first three weeks after hatching, additional factors would have to explain the continued differences in growth during the last five weeks of the current study.

Larger body size in reptiles might enhance fitness through many ecological interactions, including competitive dominance (Stamps 1984), ability to eat larger and more diverse prey (Vitt 2000), decreased predation vulnerability (Ferguson & Fox 1984; Vitt 2000), greater thermal

inertia in thermoregulation (Porter & Gates 1969; Stevenson 1985), and starvation resistance in low resource periods (Schultz & Conover 1999). Therefore, developmental conditions that affect body size can have important consequences for the evolutionary trajectories of populations. Differences among populations in these conditions, including egg incubation temperatures, could thus lead to differentiation among populations in reaction norms. The effects of incubation temperature on growth rates of juveniles differed among the three eastern populations of *A. carolinensis*. This result is not surprising considering differences in egg size, adult size, and embryonic growth and developmental rates among these populations (Michaud & Echternacht 1995; Goodman, In Press). However, this study does serve to caution those who would characterize reaction norms of growth and development in a species by experimentation in one population.

Many studies of temperature-induced plasticity in reptiles examine immediate effects only in hatchlings (reviewed in Deeming 2004; however, see O'Steen 1998; Buckley et al. 2007). However, studies must be extended beyond this life stage to determine any long-term effects that may not be initially apparent. Although different incubation temperatures did not produce initial differences in body size in hatchling *A. carolinensis*, latent effects of this developmental condition were evident in growth rates and body size at eight weeks of age. This stands in contrast to a recent, similar study with the lizard *Sceloporus undulatus*, wherein different incubation temperatures produced notable differences in morphology at hatching, but differences did not persist to seven weeks in a common environment (Buckley et al. 2007). These studies indicate that environmentally shaped traits in reptiles must be studied on a species by species basis, using multiple populations that may vary in reaction norms, and using different life stages

to understand the potential evolutionary importance of developmental conditions.

Literature cited

Alberts AC, Perry AM, Lemm JM, Phillips JA. 1997. Effects of incubation temperature and water potential on growth and thermoregulatory behavior of hatchling Cuban rock iguanas (*Cyclura nubila*). Copeia 1997:766-776.

Andrews RM. 1982. Patterns of growth in reptiles. In: Gans C, Pough FH, editors. Biology of the Reptilia, Vol 13. New York: Academic Press. p 273–320.

Angilletta MJ, Dunham AE. 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. Am Nat 162:332-342.

Angilletta MJ, Lee V, Silva AC. 2006. Energetics of lizard embryos are not canalized by thermal acclimation. Physiol Biochem Zool 79:573-580.

Atkinson D. 1994. Temperature and organism size- a biological law for ectotherms? Adv Ecol Res 25:1-58.

Avery HW, Spotila JR, Congdon JD, Fischer RU, Standora EA, Avery SB. 1993. Roles of diet protein and temperature in the growth and nutritional energetics of juvenile slider turtles, *Trachemys scripta*. Physiol Zool 66:902-925.

Berrigan D, Charnov EL. 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. Oikos 70:474-478.

Birchard GF. 2004. Effects of incubation temperature. In: Deeming DC, editor. Reptilian Incubation: Environment, Evolution, and Behaviour. UK: Nottingham Univ. Press. pp. 103-123.

Blouin-Demers G, Kissner KJ, Weatherhead PJ. 2000. Plasticity in preferred body temperature of young snakes in response to temperature during development. Copeia 2000:841-845.

Blouin-Demers G, Weatherhead PJ, Row JR. 2004. Phenotypic consequences of nest-site selection in black rat snakes (*Elaphe obsoleta*). Can J Zool 82:449-456.

Blumberg MS, Lewis SJ, Sokoloff G. 2002. Incubation temperature modulates post-hatching thermoregulatory behavior in the Madagascar ground gecko, *Paroedura pictus*. J Exp Biol 205:2777-2784.

Buckley CR, Jackson M, Youssef M, Irschick DJ, Adolph SC. 2007. Testing the persistence of phenotypic plasticity after incubation in the western fence lizard, *Sceloporus undulatus*. Evol Ecol Res 9:169-183.

Burger J. 1998. Effects of incubation temperature on hatchling pine snakes: implications for survival. Behav Ecol Sociobiol 43:11-18.

Deeming DC. 2004. Post-hatching phenotypic effects of incubation in reptiles. In: Deeming DC, editor. Reptilian Incubation: Environment, Evolution, and Behaviour. UK: Nottingham Univ. Press. p 229-251.

Downes SJ, Shine R. 1999. Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? Oecologia 120:9-18.

Elphick MJ, Shine R. 1998. Long term effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). Biol J Linnean Soc 63:429-447.

Ferguson GW, Fox SF. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. Evolution 38:342-349.

Flatt T, Shine R, Borges-Landaez PA, Downes SJ. 2001. Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. Biol J Linnean Soc 74:339-350.

Fordyce JA. 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. J Exp Biol 209:2377-2383.

Goodman RM. In Press. Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis* based on experimental manipulations of egg size. Popul Ecol.

Goodman RM, Walguarnery JW. 2007. Incubation temperature modifies neonatal thermoregulation in the lizard *Anolis carolinensis*. J Exp Zool 307A:439-448.

Gordon RE. 1960. The influence of moisture on variation in the eggs and hatchlings of *Anolis c. carolinensis* Voigt. Nat Hist Misc 173:1-6.

Iraeta P, Monasterio C, Salvador A, Diaz JA. 2006. Mediterranean hatchling lizards grow faster at higher altitude: a reciprocal transplant experiment. Funct Ecol 20:865-872.

Ji X, Chen F, Du WG, Chen HL. 2003. Incubation temperature affects hatchling growth but not sexual phenotype in the Chinese soft-shelled turtle, *Pelodiscus sinensis* (Trionychidae). J Zool 261:409-416.

Joanen T, McNease L, Ferguson MWJ. 1987. The effects of egg incubation temperature on post-hatching growth of American alligators. In: Webb GJW, Manolis SC, Whitehead PJ, editors. Wildlife management: crocodiles and alligators. Sydney: Surrey Beatty and Sons Ltd. p 533-537.

Johnston IA, Bennett AF, editors. 1996. Animals and Temperature: Phenotypic and Evolutionary Adaptation. Cambridge: Cambridge Univ. Press.

Michaud EJ. 1990. Geographic variation of life history traits in the lizard *Anolis carolinensis.* Unpubl. Ph.D. Diss., Univ. Tennessee, Knoxville.

Michaud EJ, Echternacht AC. 1995. Geographic variation in the life history of the lizard *Anolis carolinensis* and support for the pelvic constraint model. J Herpetol 29:86-97.

Niewiarowski PH, Roosenburg W. 1993. Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. Ecology 74:1992-2002.

NOAA. 1995-2004. National Climate Data Center. Climatological Data Annual Summaries: Florida Vols. 99 – 108, Tennessee Vols. 100 – 109. National Climatic Data Center, Asheville, NC.

O'Steen S. 1998. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. J Exp Biol 201:439-449.

Pigliucci M. 2001. Phenotypic Plasticity: Beyond Nature and Nurture. Baltimore, MD: Johns Hopkins Univ. Press.

Porter WP, Gates DM. 1969. Thermodynamic equilibria of animals with environment. Ecol Monogr 39:227-244.

Qualls FJ, Shine R. 1998. Geographic variation in lizard phenotypes: importance of the incubation environment. Biol J Linnean Soc 64:477-491.

Ray C. 1960. The application of Bergmann's and Allen's Rules to the poikilotherms. J Morphol 106:85-108.

Schultz ET, Conover DO. 1999. The allometry of energy reserve depletion: test of a mechanism for size-dependent winter mortality. Oecologia 119:474-483.

Shine R, Elphick MJ, Harlow PS. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. Ecology 78:2559-2568.

Sinervo B, Adolph SC. 1989. Thermal sensitivity of growth rate in hatchling *Sceloporus* lizards: environmental, behavioral and genetic aspects. Oecologia 78:411-419.

Spencer RJ, Janzen FJ, Thompson MB. 2006. Counterintuitive density-dependent growth in a long-lived vertebrate after removal of nest predators. Ecology 87:3109-3118.

Stamps JA. 1984. Rank-dependent compromises between growth and predator protection in lizard dominance hierarchies. Anim Behav 32:1101-1107.

Stevenson RD. 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. Am Nat 125:102-117.

Van Damme R, Bauwens D, Braña F, Verheyen RF. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. Herpetologica 48:220-228.

Van der Have TM, De Jong G. 1996. Adult size in ectotherms: temperature effects on growth and differentiation. J Theor Biol 183:329-340.

Vanhooydonck B, Van Damme R, Van Dooren TJM, Bauwens D. 2001. Proximate causes of intraspecific variation in locomotor performance in the lizard *Gallotia galloti*. Physiol Biochem Zool 74:937-945.

Viets BE. 1993. Lizard reproductive ecology: sex determination and parental investment. Unpubl Ph.D. Diss. Indiana Univ., Bloomington.

Vitt LJ. 2000. Ecological consequences of body size in neonatal and small-bodied lizards in the Neotropics. Herpetol Monogr 14:388-400.

Wang T, Zaar M, Arvedsen S, Vedel-Smith C, Overgaard J. 2002. Effects of temperature on the metabolic response to feeding in *Python molurus*. Comp Biochem Physiol 133:519-527.

West-Eberhard MJ. 2003. Developmental Plasticity and Evolution. New York: Oxford Univ. Press, Inc.

Appendix II:

Tables and Figures

Table II-1. Results of ANCOVAs examining effects of incubation temperature, population, and egg mass on length of incubation period, egg to hatchling mass conversion, and mass, snout vent length (SVL), body proportion, and body condition of *Anolis carolinensis* hatchlings. Eggs from MFL, NFL, and TN populations were incubated at 27 and 30 °C, while those from the LA population were incubated at 23.5, 27 and 30 °C. Factors with test statistics in italics were not significant and were removed from the model before calculating test statistics for other factors.

Factor / Covariate	**MFL, NFL, TN**			**LA**		
	df	F	P	df	F	P
Incubation period						
Temp	1, 114	738.65	< **0.001**	2, 116	895.72	< **0.001**
Population	2, 114	11.83	< **0.001**	-	-	-
Egg mass	*1, 112*	*2.26*	*0.136*	*1, 113*	*0.85*	*0.358*
Temp*Population	2, 114	17.46	< **0.001**	-	-	-
Temp*Egg mass	*1, 109*	*0.10*	*0.757*	*2, 113*	*0.40*	*0.672*
Population*Egg mass	*2, 209*	*1.78*	*0.173*	-	-	-
Egg to hatchling mass conversion (hatchling mass/egg mass)						
Temperature	*1, 113*	*0.06*	*0.813*	2, 117	1.99	0.142
Population	2, 116	8.49	< **0.001**	-	-	-
Temp*Population	*2, 113*	*0.71*	*0.496*	-	-	-
Hatchling Mass						
Temp	*1, 109*	*0.18*	*0.675*	*2, 114*	*0.78*	*0.460*
Population	2, 113	1.41	0.249	-	-	-
Egg mass	1, 113	59.45	< **0.001**	1, 118	100.80	< **0.001**
Temp*Population	*2, 109*	0.56	0.571	-	-	-
Temp*Egg mass	*1, 109*	0.33	0.568	*2, 114*	*0.56*	*0.572*
Population*Egg mass	2, 113	7.38	**0.001**	-	-	-

Table II-1. Continued

Factor / Covariate	MFL, NFL, TN			LA		
	df	F	P	df	F	P
Hatchling SVL						
Temp	*1, 109*	*0.05*	*0.830*	*2, 114*	*0.11*	*0.892*
Population	*2, 109*	*0.64*	*0.531*	-	-	-
Egg mass	1, 117	353.43	< **0.001**	1, 118	48.01	< **0.001**
Temp*Population	*2, 109*	*0.69*	*0.502*	-	-	-
Temp*Egg mass	*1, 109*	*0.02*	*0.877*	*2, 114*	*0.09*	*0.912*
Population*Egg mass	*2, 109*	*2.22*	*0.114*	-	-	-
Body proportions: TL/SVL						
Temp	*1, 109*	*1.46*	*0.230*	2, 113	1.30	0.276
Population	*2, 109*	*0.13*	*0.875*	-	-	-
Egg mass	1, 117	0.25	0.621	1, 113	0.61	0.438
Temp*Population	*2, 109*	*0.32*	*0.725*	-	-	-
Temp*Egg mass	*1, 109*	*1.26*	*0.264*	2, 113	1.51	0.226
Population*Egg mass	*2, 109*	*0.42*	*0.655*	-	-	-
Hatchling condition: mass/SVL						
Temp	*1, 109*	*0.33*	*0.567*	*2, 114*	*0.82*	*0.445*
Population	*2, 113*	*0.20*	*0.818*	-	-	-
Egg mass	1, 117	420.07	< **0.001**	1, 118	101.65	< **0.001**
Temp*Population	*2, 109*	*0.91*	*0.405*	-	-	-
Temp*Egg mass	*1, 109*	*0.58*	*0.448*	*2, 114*	*0.51*	*0.601*
Population*Egg mass	*2, 113*	*2.66*	*0.075*	-	-	-

Table II-2. Results of RM ANCOVAs examining effects of incubation temperature (27 and 30 °C), sex, population (MFL, NFL, and TN), and hatching mass or SVL on mass and SVL of *Anolis carolinensis* juveniles during eight weeks of growth in a common laboratory environment.

	Mass (to 8 weeks age)			**SVL (to 8 weeks age)**		
	df	F	P*	df	F	P*
Between subjects						
Temperature	1, 112	9.85	**0.002**	1, 112	10.75	**0.001**
Population	2, 112	3.78	**0.026**	2, 112	5.14	**0.007**
Sex	2, 112	3.15	**0.047**	2, 112	3.64	**0.029**
Hatching Mass	1, 112	68.39	< **0.001**			
Hatching SVL				1, 112	79.71	< **0.001**
Within subjects						
Time	7, 784	1.19	0.305	7, 784	2.65	0.065
Time x Temperature	7, 784	4.64	**0.012**	7, 784	0.67	0.531
Time x Population	14, 784	1.48	0.210	14, 784	2.35	**0.047**
Time x Sex	14, 784	7.62	< **0.001**	14, 784	8.78	< **0.001**
Time x Hatching Mass	7, 784	14.48	< **0.001**			
Time x Hatching SVL				7, 784	0.85	0.441

* P-values include Greenhouse-Geisser correction for sphericity.

Table II-3. Results of ANCOVAs examining effects of incubation temperature, sex, and hatchling size on the final mass and snout vent length (SVL) of *Anolis carolinensis* hatchlings from three populations (MFL, NFL, TN). Factors with test statistics in italics were not significant and were removed from the model before calculating test statistics for other factors.

Factor / Covariate	df	F	P	Factor / Covariate	df	F	P
Final Mass - MFL				**Final SVL - MFL**			
Temp	1, 23	5.64	**0.026**	Temp	1, 21	2.81	0.108
Sex	*1, 18*	*0.74*	*0.400*	Sex	1, 21	8.98	**0.007**
Hatchling mass	*1, 22*	*2.31*	*0.143*	Hatchling SVL	1, 21	6.71	**0.017**
Temp*Sex	*1, 18*	*0.44*	*0.517*	Temp*Sex	*1, 18*	*0.04*	*0.847*
Temp*Hatchling mass	*1, 21*	*2.54*	*0.126*	Temp*Hatchling SVL	*1, 18*	*0.49*	*0.494*
Sex*Hatchling mass	*1, 18*	*0.42*	*0.527*	Sex*Hatchling SVL	*1, 18*	*0.39*	*0.540*
Final Mass - NFL				**Final SVL - NFL**			
Temp	1, 46	8.25	**0.006**	Temp	1, 47	5.88	**0.019**
Sex	1, 46	3.56	0.065	Sex	*1, 45*	*3.38*	*0.073*
Hatchling mass	1, 46	9.34	**0.004**	Hatchling SVL	1, 47	7.81	**0.007**
Temp*Sex	*1, 44*	*0.06*	*0.814*	Temp*Sex	*1, 44*	*0.31*	*0.578*
Temp*Hatchling mass	1, 46	7.20	**0.010**	Temp*Hatchling SVL	1, 47	5.57	**0.022**
Sex*Hatchling mass	*1, 44*	*0.08*	*0.779*	Sex*Hatchling SVL	*1, 45*	*3.09*	*0.086*
Final Mass - TN				**Final SVL - TN**			
Temp	1, 39	4.79	**0.035**	Temp	1, 39	1.21	0.277
Sex	1, 39	4.00	0.052	Sex	1, 39	7.46	**0.009**
Hatchling mass	1, 39	7.91	**0.008**	Hatchling SVL	1, 39	5.09	**0.030**
Temp*Sex	*1, 37*	*0.70*	*0.407*	Temp*Sex	*1, 37*	*0.10*	*0.753*
Temp*Hatchling mass	*1, 37*	*0.72*	*0.402*	Temp*Hatchling SVL	*1, 37*	*0.00*	*0.961*
Sex*Hatchling mass	1, 39	5.07	**0.030**	Sex*Hatchling SVL	1, 39	7.96	**0.007**

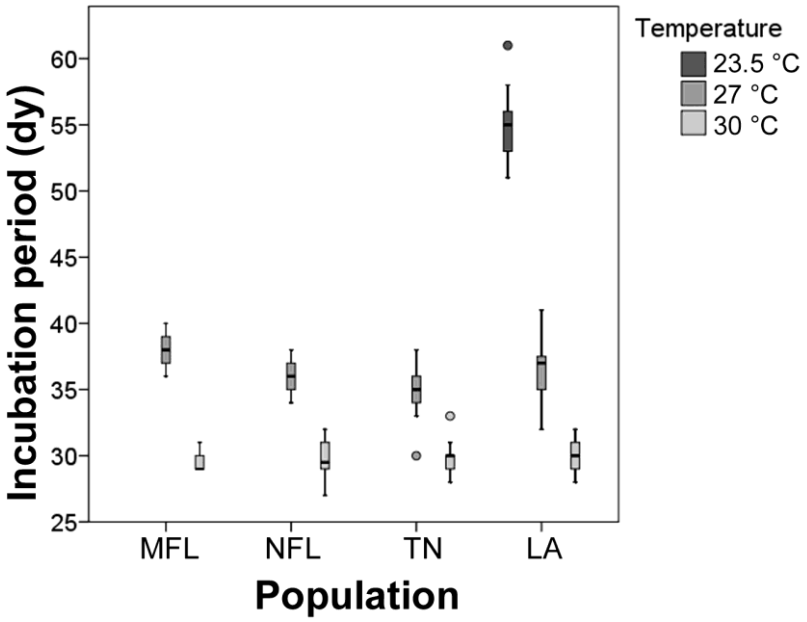

Fig II-1. Incubation periods for embryos of *Anolis carolinensis* from four populations (MFL, NFL, TN, and LA) incubated at up to three temperatures (23.5, 27 and 30 °C). Boxplot shows the median, interquartile range, and outliers for each population and treatment.

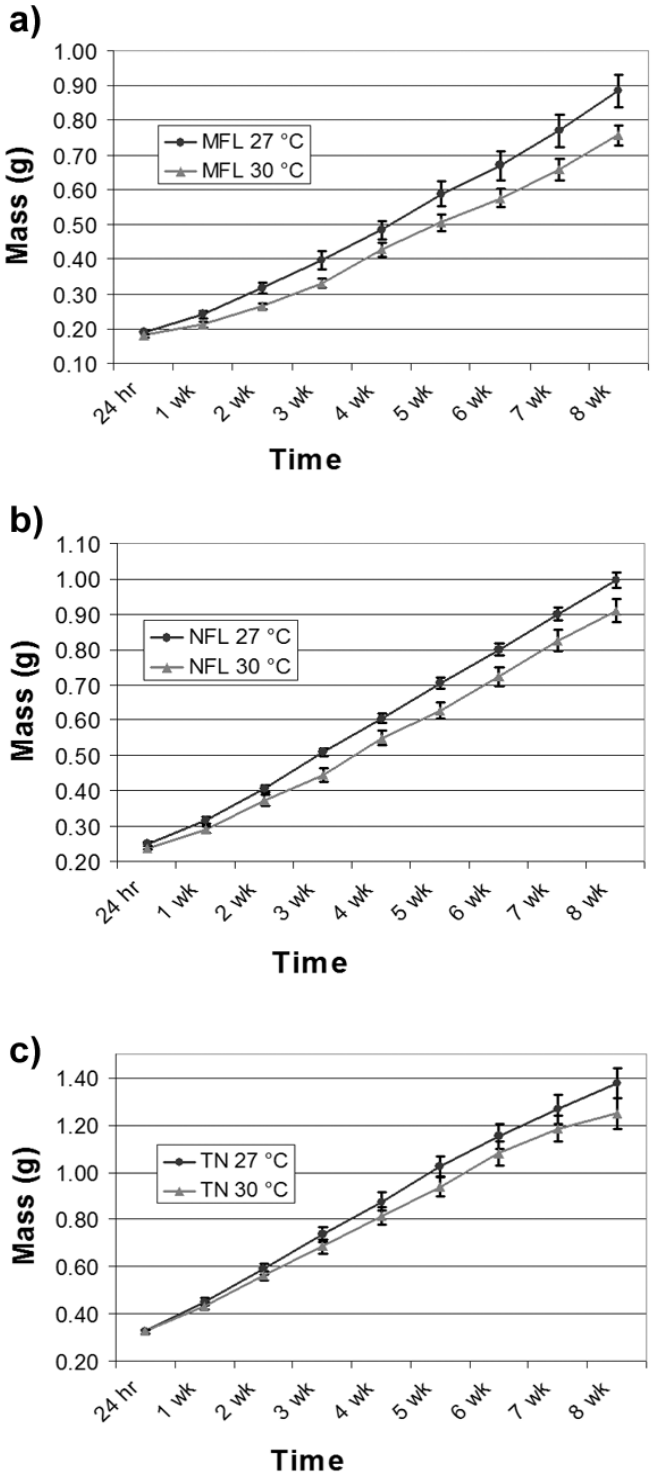

Fig II-2. Growth in mass of juvenile *Anolis carolinensis* incubated in two temperatures (27 and 30 °C) and then reared in a common laboratory environment for eight weeks. Average mass is shown for juveniles from three populations: MFL(a), NFL (b), and TN (c). Error bars are ± 1 SE.

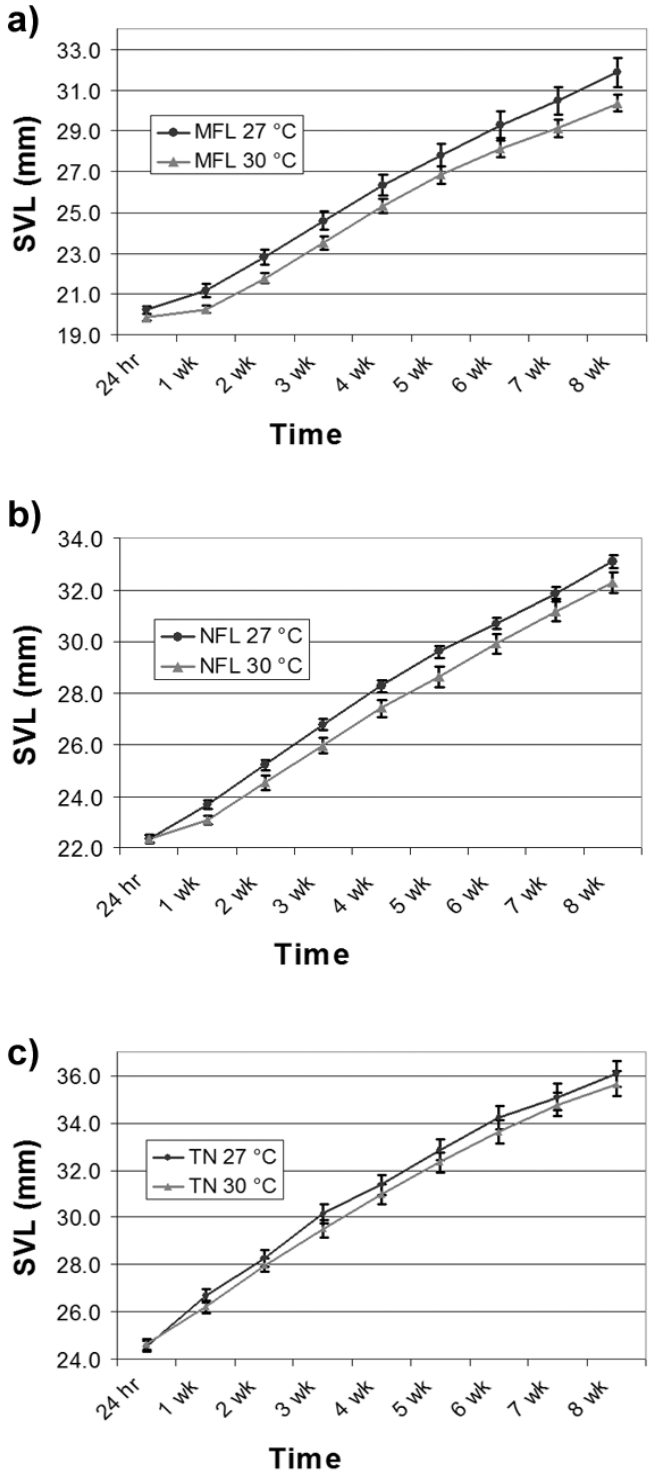

Fig II-3. Growth in snout vent length (SVL) of juvenile *Anolis carolinensis* incubated in two temperatures (27 and 30 °C) and then reared in a common laboratory environment for eight weeks. Average SVL is shown for juveniles from three populations: MFL (a), NFL (b), and TN (c). Error bars are ± 1 SE.

Part III.

Temperature-induced plasticity in an ectotherm:

the “temperature-size rule” examined at cellular and organismal levels

The following part is a slightly modified version of a manuscript currently under peer review at a scientific journal:

Goodman RM and Heah TP. (In review). Temperature-induced plasticity in an ectotherm: the “temperature-size rule” examined at cellular and organismal levels.

As the first author of this paper, I selected the topic of study and designed the study. With my co-author's involvement, I collected and compiled the data, conducted the statistical analyses, and wrote the primary text of the manuscript.

Abstract

The temperature size rule (TSR) describes a widespread pattern among ectotherms, wherein individuals reared in cooler temperatures experience slowed growth and developmental rates, but reach a larger size than those from warmer temperatures. Another reaction norm, wherein cells grow to be larger in cool environments, had been suggested to be responsible for the TSR. We demonstrated temperature-induced plasticity in erythrocytes and epithelial cells of hatchlings lizards, *Anolis carolinensis,* 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. Therefore, temperature-induced plasticity in an ectotherm applies at the cellular, but not organismal, level in *A. carolinensis*. Furthermore, reaction norms for cell size differed among populations, so we reject the hypothesis of a physiological constraint at the cellular level for the TSR. We demonstrated 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 constricted plasticity of cell size in response to variable incubation environments.

Introduction

The "temperature-size rule" (TSR) describes a widespread pattern among ectothermic vertebrates and invertebrates, and its proximate mechanisms and evolutionary explanations have interested 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

the majority of 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. 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 species. The TSR has therefore been called a life history puzzle or paradox (Berrigan & Charnov 1994; Angilletta et al. 2004).

Several mechanistic explanations have been proposed for the TSR, though none are universally accepted (reviewed in Atkinson & Sibly 1997 and in 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 growth. Theoretically, the rate of DNA replication is more temperature-dependent than the rate of protein synthesis, because the former depends on more temperature-sensitive speed of DNA polymerases whereas the latter depends on less temperature-sensitive speed of diffusion of smaller molecules (Van der Have & De Jong 1996; Jarosik et al. 2004; Walters & Hassall 2006). Reaction norms for growth of larger cells in cooler environments have been demonstrated in some ectothermic invertebrates including *Drosophila*, dung flies, crickets, and nematodes (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) found that temperature-induced plasticity varied 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).

Geographic trends in cell size and body 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 *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 TSR-related thermal reaction norms can evolve rapidly within a species in natural field conditions. Such rapid trait divergence argues 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 and in Arendt 2007). Aquatic organisms might be expected to respond differently to temperature than terrestrial organisms, because of the potential for oxygen limitation in warmer waters (Woods 1999). So far there have been no attempts to examine temperature-induced plasticity of cell size in a terrestrial, ectothermic vertebrate. Also,

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 tests whether geographic variation in cell size and plasticity for cell size exist in a terrestrial, ectothermic vertebrate, *Anolis carolinensis* (the Green Anole lizard; Polychrotidae). Specifically, we tested the null hypothesis that lizards from four populations of *A. carolinensis* show no variation in cell size and no plasticity in cell size in response to differing egg incubation temperatures. Examination of thermal reaction norms for cell size then allowed a test of the suggestion that temperature-induced plasticity of cell size constrains that of body size, explaining on a proximate level the TSR. The possibility of a physiological constraint in cell size responsible for the TSR was examined by comparing thermal reaction norms for cell size among multiple populations of *A. carolinensis*.

Materials and methods

In May and June of 2005, we collected 30-35 adult female lizards from each of four populations in the eastern range of *Anolis carolinensis*. Most females at this point in the reproductive season had already copulated and were storing sperm, which they subsequently used to fertilize eggs in the laboratory (ovulated and oviposited singly; Licht 1973). Lizards were collected from south of Greenback, Blount Co., TN (N 35° 33.486', W 84° 06.210': TN), Augusta, Columbia Co., GA (N 33° 32.976', W 82° 02.228': GA), Jacksonville, Duval Co., FL (N 30° 15.95', W 81° 30.70': North Florida- NFL), and east of Orlando, Seminole Co., FL (N

28° 37.92', W 81° 07.48': Middle Florida- MFL). Lizards were transported to the University of Tennessee, Knoxville and processed within three days of capture. Additionally, 65 adult females collected within a 100 km radius of LaPlace, Louisiana (LA; ca. N 30° 03.93', W 90° 29.18') were purchased from a reptile supplier and shipped to the university within 48 hrs. 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. Fresh blood samples were collected on slides and diluted with 0.85% NaCl solution upon clipping the distal portion of one toe from TN, GA, NFL, and MFL females. Unfortunately, epithelial cells could not be collected (nondestructively) from these individuals to compare with those of offspring as described below. Microscopy and measurement of erythrocytes are described below. Blood samples were not collected from LA adult females, and eggs from this population were subject to one additional temperature treatment. However, all other methods for housing and handling of eggs and lizards in this study were equivalent for all five populations.

Adult females were housed individually in 3.8 L glass jars with screened lids, a perch, cover object, and Repti-sand® substrate (ZooMed Laboratories, Inc.). 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 hour light:dark 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.

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 which produce relatively high survival in *A. carolinensis* (Viets 1993). The order of eggs in all treatments was distributed evenly by random assignment of the first egg of each female (and of the second egg in LA). 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; SD = 0.86) differed from those of 27 and 30 ˚C treatments (used for all populations; SD = 0.47 and 0.34˚C respectively) due to logistic 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 30 minutes 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 collected after clipping toes (used for identification in additional studies) and 5 mm of the tail. Both erythrocytes and epithelial cells were digitally photographed under 400x power microscopy immediately after collection. Mass (before toe- 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. 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 in

Scion Image© software (Scion Corporation, Frederick, MD). Average cell size was computed as the average of the visible surface area of 40 cells for each cell type.

Within wild-caught females and within all hatchlings of each population incubated at 27 °C, linear regression models were used to test for correlations between egg mass, lizard mass, or SVL and average cell size of erythrocytes or epithelial cells. Cell sizes of erythrocytes were compared among adult females from the four eastern populations using Analysis of Variance (ANOVA). All analyses that follow were restricted to one offspring per female per population and treatment. Sizes of erythrocytes and epithelial cells were compared among lab-reared hatchlings from all populations using ANOVAs with population and sex as factors. Further analyses of cell size were conducted separately for the three eastern populations that were subject to two incubation temperatures, and for the LA population that was subject to three temperatures. Following significant results, cell size within each population was analyzed using ANCOVA models. Potential factors in all appropriate analyses included temperature, population, sex, and interactions among these factors. Sample sizes for male and female hatchlings from each incubation temperature and for each population are shown in Table III-1 (all figures and tables for Part III are located in Appendix III).

In all analyses, non-significant interaction terms were dropped from the model, and results of reduced models are presented. Homogeneity of variance among groups was verified. When assumptions of normality of residuals were not met, the Kruskal-Wallis ANOVA on Ranks was substituted for ANOVA. When comparisons were reduced to one factor and two treatment groups, t tests were employed (or Mann Whitney U tests for data with distributions deviating from normality). Following significant results in ANOVA tests, Tukey Kramer

multiple comparison tests were used to compare differences among groups. All statistical analyses were performed in NCSS with a critical alpha of 0.05.

Results

Erythrocytes

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. III-1a). Within females and within lab-reared offspring from each of five populations (eggs incubated at 27 °C only), neither mass nor SVL significantly explained variation in erythrocyte size (Table III-2).

Overall, in hatchlings from the three eastern populations, cooler incubation resulted in larger erythrocytes (Table III-3). There was no direct effect of population on erythrocyte size, and a non-significant trend for an interaction effect of population and temperature (Table III-3). At the incubation temperature of 27 °C, geographic variation in erythrocyte size was evident among all five populations sampled (ANOVA: $F_{4,160} = 3.83$, $P = 0.005$; Fig. III-1b). This effect was driven primarily by the inclusion of GA, wherein erythrocytes were larger than those from NFL (other populations were intermediate).

Within MFL, erythrocytes from 27 ºC were 8 % larger in surface area than those from 30 ºC (t test- $N_{27} = 11$, $N_{30} = 14$, $t = 2.150$, $P = 0.042$, Fig. III-2a). There was no effect of temperature on cell size in NFL (t test- $N_{27} = 26$, $N_{30} = 28$, $t = 0.198$, $P = 0.844$, Fig. III-2b) or in TN (t test: $N_{27} = 24$, $N_{30} = 22$, $t = 1.26$, $P = 0.236$; Fig. III-2c). In LA, both incubation temperature and sex

affected erythrocyte size (Table III-3). Erythrocytes from 23.5 ˚C were 7-8 % larger in surface area than those from 27˚C and 30˚C (Tukey Kramer MCT, $p < 0.05$; Fig. III-2d). 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).

Epithelial Cells

Within hatchlings from all five populations (eggs incubated at 27 °C only), there were no significant correlations between hatching mass or hatching SVL and epithelial cell size (Table III-2). Cell size demonstrated geographic variation among hatchlings from all populations (ANOVA: $F_{4,104} = 3.05$, $P = 0.020$). Hatchlings from GA had larger epithelial cells than those from NFL and MFL at 27°C; other populations were intermediate (Tukey Kramer MCT, $p < 0.05$; Fig. III-1c).

Among the three eastern populations, both temperature and population affected epithelial cell size of hatchlings; there was also an interaction between population and sex (Table III-3). In MFL, both temperature and sex affected cell size (ANOVA: Temp- $F_{1, 14} = 4.62$, $P = 0.049$; Sex- $F_{1, 14} = 5.23$, $P = 0.038$). Cells from 27 ˚C were 23 % larger in surface area than those from 30˚C (Fig. III-3a). 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 (NFL: t test- $N_{27} = 25$, $N_{30} = 22$, $t = 0.628$, $P = 0.533$; TN: t test- $N_{27} = 22$, $N_{30} = 16$, $t = 1.459$, $P = 0.153$; LA: Table III-3; Figs. III-3b-d).

Conclusions

This study is the first to document both temperature-induced plasticity and a latitudinal trend in cell size in a terrestrial, vertebrate species. Wild female *Anolis carolinensis* from northern populations had larger erythrocytes than those from southern populations. Although northern females were larger, body size did not explain variation in cell size within populations. This result suggests that differences in body size among populations are not correlated with 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 an importance of environmental effects in determining erythrocyte size. Temperature-induced plasticity in erythrocyte size was also supported experimentally in this study, with erythrocytes from hatchlings incubated at cooler temperatures being larger than those from warmer temperatures. Epithelial cell size of hatchlings differed among populations; however, there was great variation within as compared to among populations and no apparent latitudinal trend in cell size.

Temperature-induced plasticity was found in some populations for both cell types under study. Cooler temperatures produced larger erythrocytes in two of five populations. An additional population (TN) showed a trend of increased cell size at 27 versus 30 °C that was not statistically significant. Perhaps the greater temperature range including 23.5 °C would be necessary to reveal plasticity in this population, as was the case for the LA population. Epithelial cells were only affected by egg incubation temperature in one out of five populations in this study. These cells were more variable in size than erythrocytes, perhaps due to 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, may 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, supporting the generality of a TSR pattern in ectotherms at the cellular level. However, 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 contributes to TSR patterns of body size.

The southernmost populations in this study (MFL and LA) demonstrated plasticity in cell size, whereas the northernmost populations (TN and GA) did not. The latter may have exhibited plasticity in cells if eggs were exposed to 23.5 ºC 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 ºC. We suggest that selection pressure for larger cell size in northern populations has constricted plasticity of cell size in response to variable incubation environments. The northern sites in this study have much colder winters with periods of low feeding activity (Jenssen et al. 1996; Bishop & Echternacht 2004), which may 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, while 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. Despite the plasticity in cell size documented here, a concurrent study demonstrated no temperature-induced plasticity exists for body size at hatching over the same range of incubation temperatures (Goodman 2008). Therefore, the factors that shape cell size on developmental and evolutionary time scales must differ from those acting on body size in *A. carolinensis*. These results contradict the suggestion that plasticity at the cellular level drives the TSR at the organismal level (Van Voorhies 1996; Blanckenhorn & Llaurens 2005), and support the assertion that selection on and regulation of size varies among scales within an organism. Although cells, organs, and bodies may grow larger in cooler environments, the explanations of these patterns may not fit into one simple and general explanation (Angilletta & Dunham 2003; Atkinson et al. 2006).

Literature cited

Angilletta MJ, Dunham AE. 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. Am Nat 162:332-342.

Angilletta MJ, Steury TD, Sears NW. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Int Comp Biol 44:498-509.

Arendt J. 2007. Ecological correlates of body size in relation to cell size and cell number: patterns in flies, fish, fruits and foliage. Biol Rev 82:241-256.

Arendt J, Hoang L. 2005. Effect of food level and rearing temperature on burst speed and muscle composition of Western Spadefoot Toad (*Spea hammondii*). Funct Ecol 19:982-987.

Atkinson D. 1994. Temperature and organism size- a biological law for ectotherms? Adv Ecol Res 25:1-58.

Atkinson D, Morley SA, Hughes, RN. 2006. From cells to colonies: at what levels of body organization does the 'temperature-size rule' apply? Evol Dev 8:202-214.

Atkinson D, Sibly RM. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends Ecol Evol 12:235-239.

Azevedo RBR, French V, Partridge L. 2002. Temperature modulates epidermal cell size in *Drosophila melanogaster*. J Insect Physiol 48:231-237.

Berrigan D, Charnov EL. 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. Oikos 70:474-478.

Bishop DC, Echternacht AC. 2004. Emergence behavior and movements of winter-aggregated green anoles (*Anolis carolinensis*) and the thermal characteristics of their crevices in Tennessee. Herpetologica 60:168-177.

Blanckenhorn WU, Llaurens V. 2005. Effects of temperature on cell size and number in the yellow dung fly *Scathophaga stercoraria*. J Thermal Biol 30:213-219.

Goodman RM. 2008. Latent effects of egg incubation temperature on growth in the lizard *Anolis carolinensis*. J Exp Zool 309A:1–9.

James AC, Azevedo RBR, Partridge L. 1995. Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. Genetics 140:659-666.

James AC, Azevedo RBR, Partridge L. 1997. Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. Genetics 146:881-890.

Jarosik V, Kratochvil L, Honek A, Dixon AFG. 2004. A general rule for the dependence of developmental rate on temperature in ectothermic animals. Proc Royal Soc Lond B 271:S219-S221.

Jenssen TA, Congdon JD, Fischer RU, Estes R, Kling D, Edmands S, Berna H. 1996. Behavioural, thermal, and metabolic characteristics of a wintering lizard (*Anolis carolinensis*) from South Carolina. Funct Ecol 10:201-209.

Karl I, Fischer K. 2008. Why get big in the cold? Towards a solution to a life history puzzle. Oecologia 155:215-225.

Kingsolver JG, Massie KR, Ragland GJ, Smith MH. 2007. Rapid population divergence in thermal reaction norms for an invading species: breaking the temperature-size rule. J Evol Biol 20:892-900.

Licht P. 1973. Influence of temperature and photoperiod on annual ovarian cycle in lizard *Anolis carolinensis*. Copeia 1973:465-472.

Litzgus JD, DuRant SE, Mousseau TA. 2004. Clinal variation in body and cell size in a widely distributed vertebrate ectotherm. Oecologia 140:551-558.

Nijhout HF. 2003. The control of body size in insects. Dev Biol 21:1-9.

Partridge L, Barrie B, Fowler K, French V. 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. Evolution 48:1269-1276.

Ray C. 1960. The application of Bergmann's and Allen's Rules to the poikilotherms. J Morphol 106:85-108.

Szarski H. 1983. Cell size and the concept of wasteful and frugal evolutionary strategies. J Theor Biol 105:201-209.

Van der Have TM, De Jong G. 1996. Adult size in ectotherms: temperature effects on growth and differentiation. J Theor Biol 183:329-340.

Van Voorhies WA. 1996. Bergmann size clines: a simple explanation for their occurrence in ectotherms. Evolution 50:1259-1264.

Viets BE. 1993. Lizard reproductive ecology: sex determination and parental investment. Unpubl. Ph.D. Diss., Indiana Univ., Bloomington.

Walters RJ, Hassall M. 2006. The temperature-size rule in ectotherms: may a general explanation exist after all? Am Nat 167:510-523.

Woods HA. 1999. Egg-mass size and cell size: Effects of temperature on oxygen distribution. Am Zool 39:244-252.

Appendix III:

Tables and Figures

Table III-1. Sample sizes for lab-reared hatchlings of *Anolis carolinensis* used in analyses of geographic variation and plasticity of cell size. Numbers of males and females (M and F) are indicated for each of three incubation temperatures and each of four populations (LA, MFL, NFL, TN, see text for details).

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

Table III-2. Regression equations for cell size (surface area in μm^2) on mass and snout vent length (SVL) of wild-caught adult females from four populations (MFL, NFL, TN, GA) and laboratory-reared hatchlings of these females plus one additional population (LA). Sample size (N) and coefficients of determination (R^2) are presented, along with t-values and P-values for t tests that slope is equal to zero.

	Erythrocyte Average SA & Mass (g)					Erythrocyte Average SA & SVL (cm)				
Adult Females	**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 & Mass (g)					Erythrocyte Average SA & SVL (mm)				
Lab Hatchlings	**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
TN	y = 163.1 - 22.9 x	58	-1.026	0.309	0.02	y = 168.7 - 0.5 x	58	-0.549	0.585	<0.01
LA	y = 144.9 + 52.6 x	32	1.3902	0.175	0.06	y = 113.0 + 2.0 x	32	1.0589	0.298	0.04

	Epithelial Cell Average SA & Mass (g)					Epithelial Cell Average SA & SVL (mm)				
Lab Hatchlings	**Equation**	**N**	**t**	**P**	**R^2**	**Equation**	**N**	**t**	**P**	**R^2**
MFL	y = 625.0 + 1726.7 x	9	0.3872	0.710	0.02	y = 3090.7 - 106.3 x	9	-0.877	0.410	0.10
NFL	y = 895.5 + 384.5 x	25	0.3707	0.714	<0.01	y = 1512.5 - 23.2 x	25	-0.652	0.521	0.02
GA	y = 889.0 + 590.1 x	45	1.5377	0.132	0.05	y = 418.5 + 27.3 x	43	1.671	0.102	0.06
TN	y = 1034.8 + 35.8 x	49	0.1012	0.920	<0.01	y = 1031.6 + 0.6 x	49	0.0392	0.969	<0.01
LA	y = 922.7 + 455.2 x	28	0.7708	0.448	0.02	y = 427.0 + 25.9 x	28	0.8906	0.381	0.03

Table III-3. Results of ANOVAs comparing cell size (average surface area in μm^2) among lab-reared hatchlings of *Anolis carolinensis* from different populations, of different sexes, and from eggs incubated at different temperatures (27 and 30 °C for MFL, NFL, TN; 23.5, 27 and 30 °C for LA). F-values, P-values, and degrees of freedom (df) are shown. Non-significant factors and interaction terms were omitted, and results of reduced models are presented.

Factor / Covariate	df	F	P
Erythrocytes MFL, NFL, TN			
Temp	1, 119	6.03	**0.016**
Population	2, 119	0.63	0.535
Temp*Population	2, 119	2.8	0.064
Erythrocytes- LA			
Temp	2, 114	15.1	**<0.001**
Sex	1, 114	5.33	**0.022**
Epithelial Cells- MFL, NFL, TN			
Temp	1, 97	4.93	**0.029**
Population	2, 97	4.26	**0.017**
Sex	1, 97	1.56	0.214
Population*Sex	2, 97	4.94	**0.009**
Epithelial Cells- LA			
Temp	2, 105	1.85	0.163

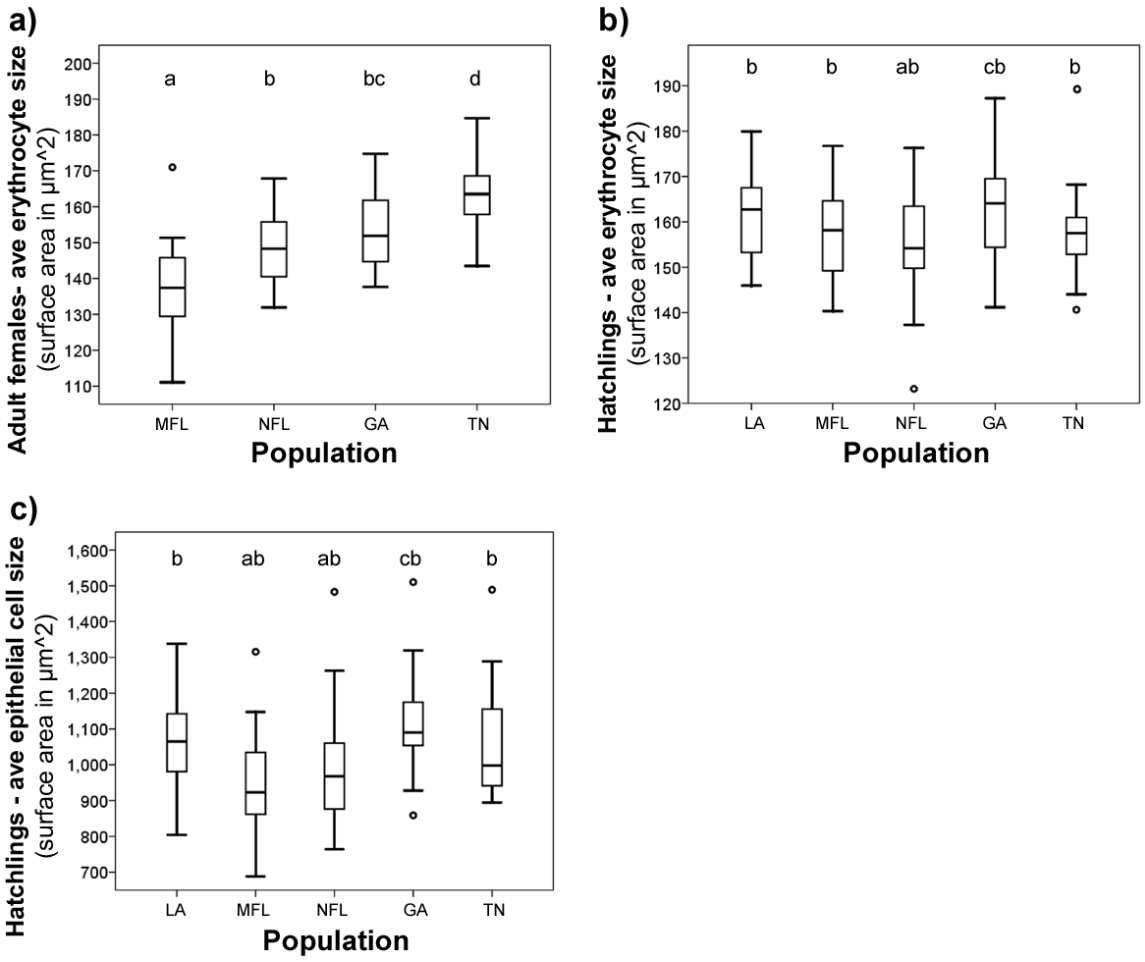

Figure III-1. Average cell sizes (surface area in µm2) of (a) erythrocytes of wild, adult female *Anolis carolinensis*, (b) erythrocytes of laboratory-reared offspring from females in five populations (LA, MFL, NFL, GA, TN), and (c) epithelial cells of the laboratory-reared offspring. Boxplots shows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

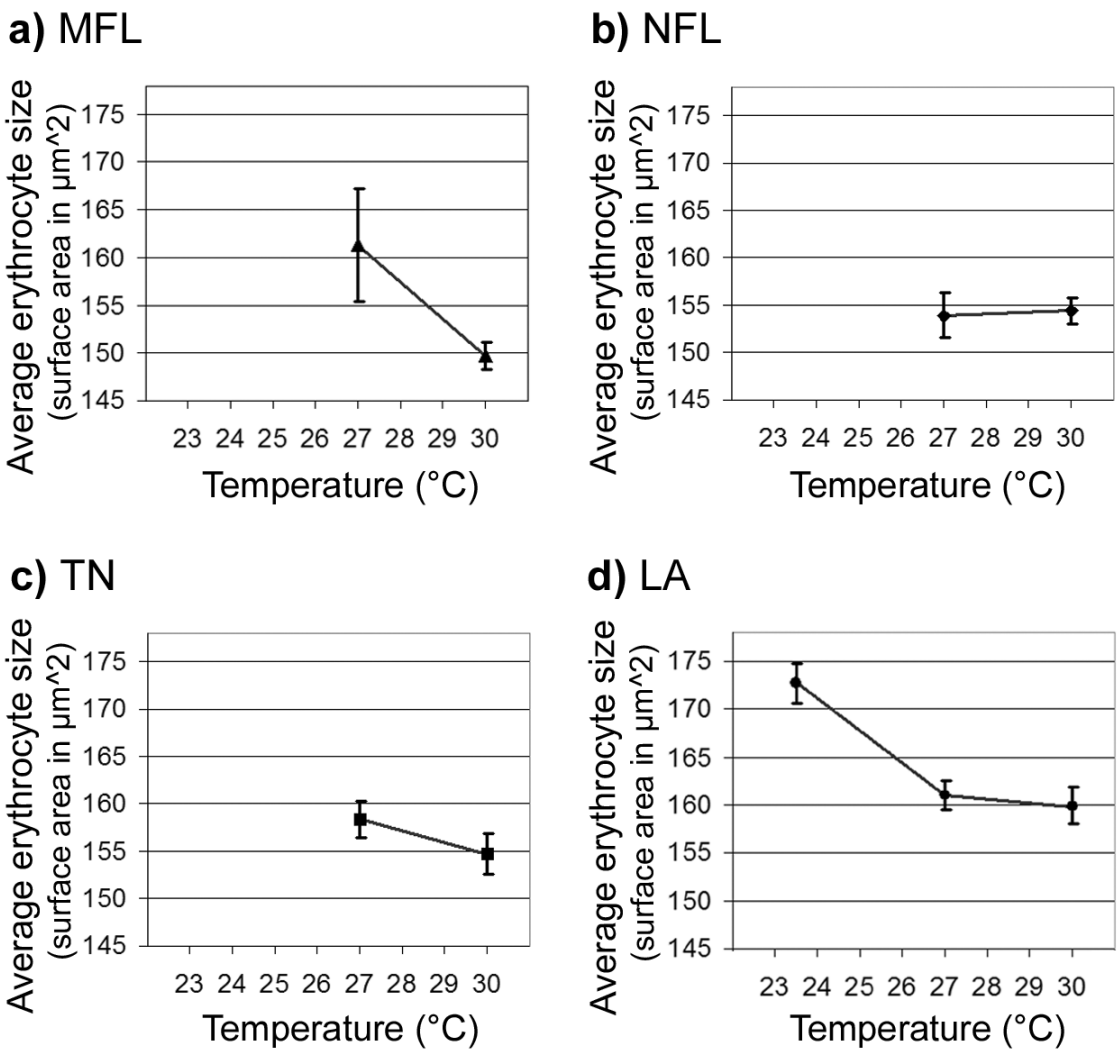

Figure III-2. Average erythrocyte sizes (surface area in µm2) of laboratory-reared hatchling *Anolis carolinensis* incubated at different temperatures. Hatchlings came from eggs collected from wild-caught females from four populations (MFL, NFL, TN, LA; see text for details). Error bars denote ± 1 SE.

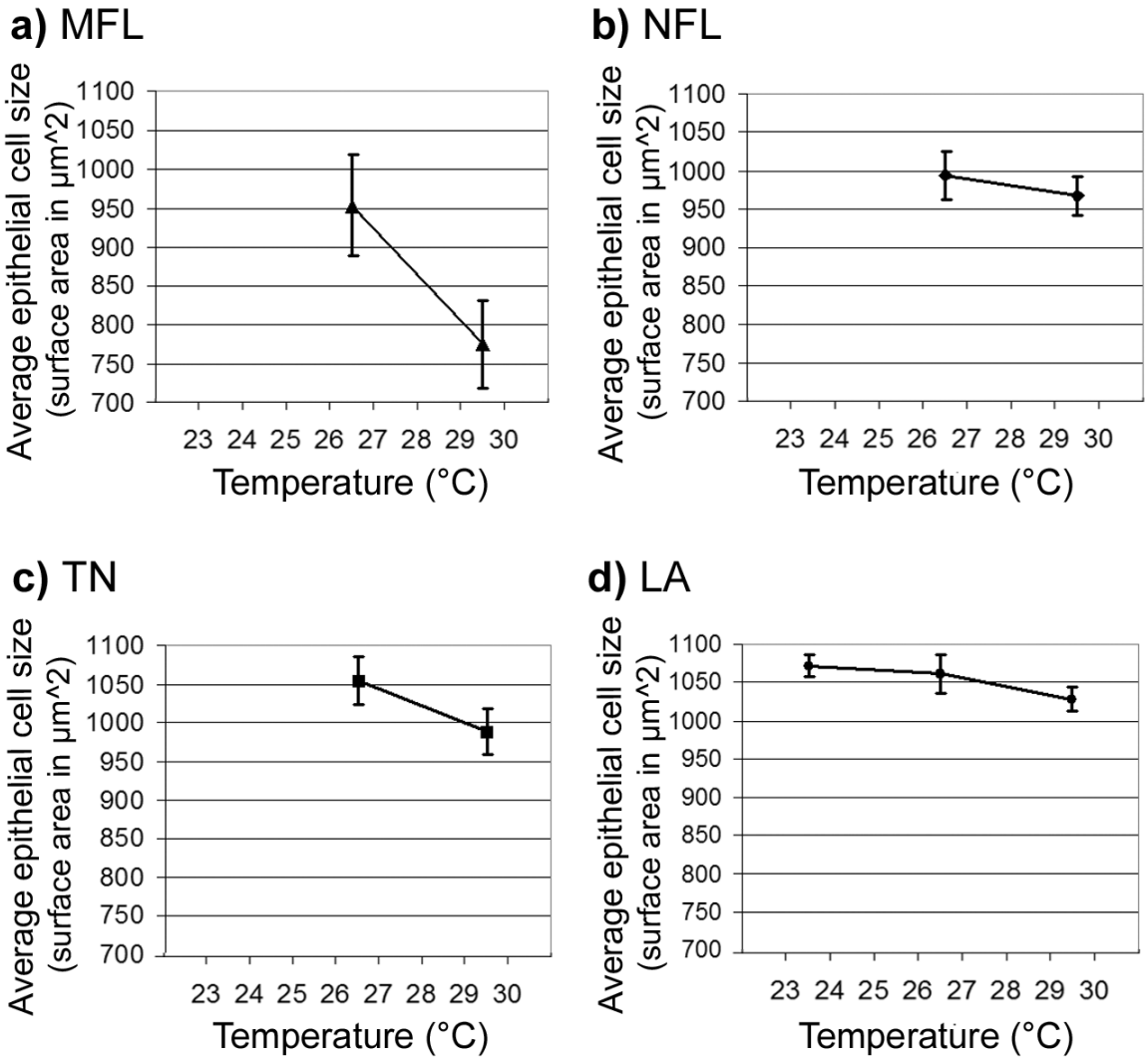

Figure III-3. Average epithelial cell sizes (surface area in µm2) of laboratory-reared hatchling *Anolis carolinensis* incubated at different temperatures. Hatchlings came from eggs collected from wild-caught females from four populations (MFL, NFL, TN, LA; see text for details). Error bars denote ± 1 SE.

Part IV.

Do patterns of cell size and body size in an ectothermic vertebrate reflect geography and climatic gradients?

The following part will be submitted as a modified manuscript for peer review at a scientific journal:

Goodman RM, Echternacht AC, Hall JC, Welch JJ, Deng LD. Do patterns of cell size and body size in an ectothermic vertebrate reflect geography and climatic gradients?

As the first author of this paper, I selected the topic of study. I co-designed the study with Hall. I collected and compiled the data with Welch and Deng. With my co-authors' involvement, I conducted the statistical analyses and wrote the primary text of the manuscript.

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 across the native range of the green anole lizard, *Anolis carolinensis*. This species demonstrated a longitudinal pattern in body size, driven by small body size in Florida populations. If these were excluded, a latitudinal trend opposing Bergmann's Rule became evident with larger anoles in southern, warmer, and less seasonal environments. Number of muscle cells was negatively and positively related to latitude and longitude, respectively. Muscle and red blood cells were larger in western populations. These trends were driven by smaller size of both cell types in Florida. Muscle cell size also had a negative relationship with latitude whether Florida populations were included or not. Red blood cell size was also related to latitude and weakly to precipitation, but these relationships driven entirely by the Florida populations which have small cells and tend to have high levels of month-to-month variation in precipitation. Smaller body size and cell sizes in Florida anoles may be attributable to the geological history of the peninsular state or unique ecological factors in this area. The current observational study does not separate the relative effects of fixed and plastic differences among populations contributing to georaphic trends in body size and cell size.

Introduction

Biologists have long been fascinated with ecogeographic patterns in body size, such as inter- and intraspecific trends of body size associated with latitude, elevation, or environmental and climatic variables. The well-known Bergmann’s rule notes the tendency for larger-bodied

endothermic vertebrates to occur in cooler climates (Bergmann 1847). The rule was originally intended 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). Recent studies show that mammals and birds generally follow Bergmann's rule, with larger animals within a species found at higher latitudes and lower temperatures (Ashton et al. 2000; Ashton 2002a; Meiri & Dayan 2003). Explanations for larger size in endotherms at higher latitudes include fasting endurance through long winters and minimization of surface area relative to volume for heat conservation (Searcy 1980; reviewed in Cushman et al. 1993; Blackburn et al. 1999; Blackburn et al. 1999; but see Meiri et al. 2005). Among ectothermic vertebrates, these explanations should not apply, and in fact, intraspecific patterns of body size with latitude and temperature vary across taxa. Studies of amphibians and fishes produce mixed results, with some taxa but not others conforming to Bergmann's rule (Gilligan 1991; 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 (Ashton & Feldman 2003). Ashton and Feldman suggested 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 this 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 ectothermic 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; Warner & Andrews 2002; Shine 2004).

Ecogeographic trends in cell size have also been documented in several ectotherms, though 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; Atkinson & Sibly 1997). 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 (Goniakowska 1973; Szarski 1983; Mongold & Lenski 1996; 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 faster metabolism and should be able to divide more quickly, leading to faster development (Szarski 1985). Van der Have & de Jong (1996) suggested 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 four 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). Also, temperature may affect cell types differently (e.g. actively dividing cells versus differentiated cells) (Cuadrado et al. 1989; Atkinson 1994). Muscle cell 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 (Johnston et al. 1996; Arendt 2000; Arendt & Hoang 2005). Specialized wing and eye cells have been used in studies of invertebrates (reviewed in Arendt 2007); these methods are not transferable to vertebrate ectotherms. Epidermal cells from preserved carapace pieces were used in a study on variation in body size and cell size of a turtle (Litzgus et al. 2004), and shed skin cells have been used in a study of cell size and polyploidy in amphibians (Licht & Bogart 1987).

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

approximately 22º longitude and 10º degrees 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 living for more than one reproductive season. Anoles are active to some extent throughout the year and never enter full hibernation, even in areas where populations experience cold winters. In one 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 and Echternacht 2004).

Anolis carolinensis has a seasonal reproductive cycle; mating occurs in April or May through July or August, with some variation among populations (reviewed in Minesky 1999). Green anoles exhibit sexual size dimorphism. Males and females are the same size at hatching, but males grow more rapidly than females thereafter (Michaud 1990; Goodman In Press). A trend of increasing female body size with latitude was documented by Michaud and Echternacht (1995) for eight populations of *A. carolinensis* in the eastern part of its range. However, this trend may or may not apply throughout the entire geographic range. A recent study confirmed this eastern trend in body size and also documented a trend for larger RBCs with increasing latitude among females sampled from four populations (Goodman & Heah In Review). The goals of current study were to investigate potential patterns in body size and cell size across the full range of the species and to determine whether any such patterns are explained by variation in climatic conditions and latitude and longitude. Adults were collected at the beginning of the mating season when they are active and readily captured, and when most individuals are fully grown and in their first, and probably only, year of reproduction. Both RBCs from fresh blood samples and skeletal muscle cells 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 four eastern populations (Goodman In Review) 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 17 populations of *Anolis carolinensis* throughout the southeastern United States (Fig. IV-1; all figures and tables for Part IV are located in Appendix IV) in May-June of 2006 and of 2007. Collection sites included various natural and human-modified habitats, but were limited to areas that did not have any artificial sources of water (sprinklers, irrigation, etc.). We were only able to collect 10 and 17 lizards from populations in BV_TX and 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 on a multi-year drought). Axtell (2005) suggested that the western extent of the species range expanded within the 20th century due to commensalism with humans (also see range map expansion between Conant 1958 and Conant & Collins 1998). *Anolis carolinensis* may be periodically restricted to water-rich habitats (often via human supply) in the driest and western-most portion of the range.

Lizards were measured for mass (to .001 g) and snout vent length (SVL, to 0.5 cm) within 48 hours of collection. Also, blood samples were taken on a slide after clipping one toe,

diluted with 0.85% NaCl buffer, and covered with a cover slip. Digital images of blood samples were immediately taken under 40 X microscopy. Lizards were euthanized via inhalation of isoflourane and then preserved in 10% formalin.

Numbered grids were added to digital images of red blood cells (RBCs), to aid in random selection of cells for measurement. Ten cells from each of four images were measured for each lizard using Scion Image© software (Scion Corporation, Frederick, MD). Average cell size was computed as the surface area (in 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 a HP Faxitron 43805N machine with metal pins inserted through the cloaca. Radiographs were taken with 10 second exposures at 40kVp using Kodak Biomax XAR film. X-rays were converted to digital images using a scanner, and the distance from the metal pin to the top of the tenth caudal vertebra was measured using Scion Image software. A metal standard was placed in all x-rays in both years to ensure calibration.

Tails were cut from lizards using a razor blade at a point measured minus 1 cm from the top of the tenth caudal vertebra, as determined individually for each lizard based on radiograph measurements. We initially froze on dry ice, then directly mounted (using cryostat mounting medium) a section of tail with the initial cut face-up. Transverse sections of 50 um thickness were taken with a cryostat from the initial cut above the tenth 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 X magnification as needed based on section size. Sections were taken from the anterior to 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 IV-2 shows a sample of one of these image, with the four 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 four muscle segments reached their maximum size (just prior to the image in which these muscles opened up and joined with the neighboring muscles) was chosen for data collection. Cross-sectional surface area of the four 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 muscle cells (hereafter MC number) to yield an estimate of cross-sectional surface area of muscle cells (in 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 (http://www.ncdc.noaa.gov; National Climatic Data Center, Asheville, NC) for a 20-year period (1986-2006 and 1987-2007 for populations collected in 2006 and 2007, respectively). Weather data were usually taken from the weather station closest to a given collecting locality. If, however, these data were incomplete, the missing data were obtained from the next nearest station that recorded the data (see Table 1).

For each month, the lowest recorded temperature, total precipitation, and average mean, maximum, minimum, and dewpoint temperatures were calculated. These estimates were then used to calculate the following historical estimates: annual average of lowest recorded temperature throughout each year (among years), annual averages and variances (first among

months within years, then among years) for average mean, maximum, minimum, and dewpoint temperatures, and annual total and variances (first among months within years, then among years) of precipitation.

Statistical analyses

Due to high levels of multicollinearity between climatic variables, principles component analysis in JMP 7.0 (SAS Institute, Inc., Cary, NC) was used to reduce the 11 climatic variables prior to regression analysis. PCA resulting in two principle components explaining 95.6% of original variation. One explained 79.9% of variation and was associated positively with average maximimum, minimum, mean, and dewpoint and absolute minimum temperatures, and negatively with annual variance in all temperatures (PC_{temp}). The second principle component explained 15.7% of variation and was positively associated with average and variance in precipitation (PC_{precp}).

Averages within the 19 populations were estimated for body size (summaries for all populations are in Table IV-2), MC size and number, and RBC size. These response variables were then modeled with potential predictor variables of latitude, longitude, PC_{temp}, and PC_{precp} using PROC REG in SAS 9.1 (SAS Institute, Inc., Cary, NC). 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. Latitude and PC_{temp} were the only two of four 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 two variables (partial t tests of slope and semi-partial or SP r^2)

within a model could not be separated statistically (Stillwell 2007).

Within populations, analysis of variance (ANOVA) and regression analysis were used to determine if there was sexual dimorphism in or effect of body size (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 ANOVAs, and homoscedasticity and normality of error terms were verified for regressions.

Results

Among populations

Longitude was the only variable in the best-fit model for body size (SVL) in *Anolis carolinensis* based on AIC (model: AIC = 46.6, $r^2 = 0.476$, $F = 15.43$, df = 1, 17, $p = 0.001$; longitude: slope = 0.462 ± 0.118). Anoles were larger in the western part of their range (Fig. IV-3). Because body size in Florida appeared notably smaller than in the rest of the range, additional analysis was conducted for populations excluding Florida. Exclusion of the five 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 body size (SVL) without Florida populations included only latitude and PC_{precp} and explained more variation than the previous model for all populations (AIC = 16.06, $r^2 = 0.803$, $F = 22.44$, 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 PC_{precp} (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 PC_{precp} (SP r^2 = 0.002; *slope* t = -0.32, p = 0.755), suggesting that longitude does not influence body size outside of Florida.

The AIC best-fit model for MC size included latitude and longitude (model: r^2 = 0.457, AIC = 210.8; F = 6.72, df = 2, 16, p = 0.008). The effect of latitude was marginally non-significant when modeled with the effect of longitude *(*latitude: SP r^2 = 0.242, slope = -38.75 ± 18.57, t = -2.09, df = 1, 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. IV-4).

The best-fit model for MC number also included latitude and longitude (model: r^2 = 0.557, F = 10.07, df = 2, 16, p = 0.002). Tail muscles contained more skeletal muscle cells 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. IV-5). Exclusion of Florida populations resulted in a lack of correlation between cell number and either latitude or longitude (model: r^2 = 0.074, F = 0.44, df = 2, 11, p = 0.656; latitude: SP r^2 = 0.058, *slope* t = -0.27 p = 0.793; longitude: SP r^2 = 0.015, *slope* t = 0.43, p = 0.677), suggesting that Florida anoles drive trends in MC number.

The AIC best-fit model for RBC size included latitude, longitude, and PC_{precp} (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), and more northern populations had larger RBCs (latitude: SP r^2 = 0.441, slope = 2.87 ± 0.46, t = 6.23, p < 0.001; Fig. IV-6). Populations with larger RBCs tended to have lower values of PC_{precp} (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 analysis, all of these relationships were voided (model: $r^2 = 0.163$, $F = 0.65$, $df = 3, 10$, $p = 0.600$; latitude: SP $r^2 = 0.149$; *slope* $t = 1.09$, $p = 0.302$; longitude: SP $r^2 = 0.015$; *slope* $t = 0.41$, $p = 0.689$; PC_{precp}: SP $r^2 < 0.001$; *slope* $t < -0.01$, $p = 0.997$).

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-values > 0.05).

Conclusions

In contrast to previous studies, we found that *Anolis carolinensis* does not follow Bergmann's Rule. Michaud & Echternacht (1995) found increasing size with latitude in eight populations, and Goodman (In Press) confirmed this trend among four populations of *A. carolinensis*; however these studies were both limited to eastern populations. The current study showed a longitudinal pattern in body size, driven by small body size in Florida populations of *A. carolinensis*. When these were excluded, a latitudinal trend opposing Bergmann's Rule became 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).

Genetic relationships among populations of *A. carolinensis* are unknown, and so there may be historical factors explaining smaller body size in Florida. The first fossil of *Anolis* in North America comes from the late Miocene, and fossils of *Anolis carolinensis* in the southeastern U.S. including Florida date to the Pleistocene epoch (Auffenberg 1956; Holman 1995). Particularly during the Pleistocene, the peninsular state 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 isolated on small islands. Local body size adjustments during these times may be reflected in size patterns observed today.

Smaller body size in Florida anoles may also be attributable to ecological factors in this area**.** *Anolis carolinensis* occupies a unique niche within the southeastern range, and does not have any native competitors that occupy the same or similar microhabitats. However, the recent introduction of a congeneric competitor occurred in Florida during the last century. The introduced congener occupies a trunk- ground niche, which overlaps more closely with the trunk-crown niche of *A. carolinensis* than any native species. *Anolis sagrei* was first reported in the United States in the Florida Keys (Garman 1887), mainland populations likely appeared in the 1940's (Lee 1985; first published record is Oliver 1950), and the species is now widespread throughout Florida. Therefore, the contact time between native mainland populations of *A. carolinensis* and introduced *A. sagrei* ranges from almost 70 years to less than about five years, equivalent to about 5 - 70 generations, respectively, of *A. carolinensis*. Schoener (1970) suggested 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 the southeastern United States 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. Again, these trends were driven by smaller size of both cell types in Florida populations. MC size had a negative relationship with latitude whether Florida populations were included or not. RBC size was related to latitude and weakly to precipitation, but these relationships 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. Number of MCs was negatively and 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 United States, 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 multiple cell types when studying geographic variation in cell size.

MC 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 1) the structure and orientation of cells allowed comparison of comparable developmental units in lizards of differing body sizes, and 2) 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 (reviewed in Martin & Avery 1998) including *A. carolinensis* (Gillis 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 cannot determine how the interaction between variable environments and gene expression patterns produces the trends in body size and cell size in *A. carolinensis.* 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* (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 In Review; 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.

Literature cited

Adams DC, Church JO. 2008. Amphibians do not follow Bergmann's rule. Evolution 622:413-20.

Arendt JD. 2000. Allocation of cells to proliferation vs. differentiation and its consequences for growth and development. J Exp Zool 288: 219-234.

Arendt JD. 2007. Ecological correlates of body size in relation to cell size and cell number: patterns in flies, fish, fruits and foliage. Biol Rev Camb Philos Soc 82: 241-256.

Arendt JD, Hoang L. 2005. Effect of food level and rearing temperature on burst speed and muscle composition of Western Spadefoot Toad (*Spea hammondii*). Funct Ecol 19: 982-987.

Ashton KG, Tracy MC, de Queiroz A. 2000. Is Bergmann's rule valid for mammals? Am Nat 156: 390-415.

Ashton KG. 2002a. Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. Global Ecol Biogeogr 11: 505-523.

Ashton KG. 2002b. Do amphibians follow Bergmann's rule? Can J Zool 80: 708-716.

Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. Evolution 57: 1151-1163.

Atkinson D. 1994. Temperature and organism size- a biological law for ectotherms? Adv Ecol Res 25: 1-58.

Atkinson D, Sibly RM. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends Ecol Evol 12: 235-239.

Axtell RW. 2005. Interpretive Atlas of Texas Lizards. No. 29. *Anolis carolinensis*. Privately printed. Southern Illinois Univ., Edwardsville.

Auffenberg W. 1956. Additional records of Pleistocene lizards from Florida. J Florida Acad Sci 19: 157-167.

Belk, MC, Houston DD. 2002. Bergmann's rule in ectotherms: a test using freshwater fishes. Am Nat 160: 803-808.

Bergmann C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Göttinger Studien 3: 595-708.

Bishop DC, Echternacht AC. 2004. Emergence behavior and movements of winter-aggregated green anoles (*Anolis carolinensis*) and the thermal characteristics of their crevices in Tennessee. Herpetologica 60: 168-177.

Blackburn TM, Gaston KJ, Loder N. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. Divers Distrib 5: 165-174.

Conant R. 1958. A Field Guide to Reptiles and Amphibians of Eastern and Central North America (Series: Peterson Field Guides). Houghton Mifflin Co, Boston.

Conant R, Collins JT. 1998. A Field Guide to Reptiles and Amphibians of Eastern and Central North America (Series: Peterson Field Guides). Third ed, expanded. Houghton Mifflin Harcourt, Boston.

Cuadrado A, Navarrete MH, Canovas JL.1989. Cell size of proliferating plant cells increases with temperature: implications in the control of cell division. Exp Cell Res 185: 277-282.

Cushman JH, Lawton JH, Manly BFJ. 1993. Latitudinal patterns in European ant assemblages: variation in species richness and body size. Oecologia 95: 30-37.

Garman S. 1887. On West Indian Iguanidae and on West Indian Scincidae in the collection of the Museum of Comparative Zoology at Cambridge, Mass., USA. Bull Essex Inst 19: 25-50.

Gilligan MR. 1991. Bergmann ecogeographic trends among triplefin blennies (Teleostei: Tripterygiidae) in the Gulf of California, Mexico. Env Biol Fishes 31: 301-305.

Goniakowska L. 1973. Metabolism, resistance to hypotonic solutions, and ultrastructure of erythrocytes of five amphibian species. Acta Biol Cracov Ser Zool 16: 113-134.

Goodman RM. 2008. Latent effects of egg incubation temperature on growth in the lizard *Anolis carolinensis*. J Exp Zool 309A: 1–9.

Goodman RM. (In press). Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis* based on experimental manipulations of egg size. Pop Ecol.

Goodman RM, Heah TP. (In review). Temperature-induced plasticity in an ectotherm: the "temperature size rule" examined at cellular and organismal levels.

Gordon RE. 1956. The biology and biodemography of *Anolis carolinensis carolinensis* Voigt. Unpubl Ph.D. Diss. Tulane Univ., New Orleans, Louisiana.

Gregory TR. 2000. Nucleotypic effects without nuclei: genome size and erythrocyte size in mammals. Genome 43: 895-901.

Gillis GB, Bonvini L, Irschick DJ. 2009. Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis*. J Exp Biol 212: 604-609.

Holman, JA. 1995. Pleistocene Amphibians and Reptiles in North America. Oxford Univ. Press, New York.

James FC. 1970. Geographic size variation in birds and its relationship to climate. Ecology 51: 365-390.

Johnston IA, Vieira VLA, Hill J. 1996. Temperature and ontogeny in ectotherms: muscle phenotype in fish. In: Animals and Temperature: Phenotypic and Evolutionary Adaptation, p. 153-181. Johnston IA, Bennett AF, Eds. Cambridge Univ. Press.

King FW. 1966. Competition between two south Florida lizards of the genus *Anolis*, Unpubl Ph.D. Diss. Univ. Miami, Coral Gables, Florida.

Kolbe JJ, Losos JB. 2005. Hind-limb length plasticity in *Anolis carolinensis*. J Herpetol 39: 674-678.

Kozlowski J, Konarzewski M, Gawelczyk AT. 2003. Cell size as a link between noncoding DNA and metabolic rate scaling. PNAS 100: 14080-14085.

Lee JC. 1985. Anolis *sagrei* in Florida: Phenetics of a colonizing species. I. Meristic characters. Copeia 1985: 182-194.

Licht LE, Bogart JP. 1987. Comparative size of epidermal cell nuclei from shed skin of diploid, triploid and tetraploid salamanders (genus *Ambystoma*). Copeia: 284-290.

Litzgus JD, DuRant SE, Mousseau TA. 2004. Clinal variation in body and cell size in a widely distributed vertebrate ectotherm. Oecologia 140: 551-558.

Mayr E. 1956. Geographical character gradients and climatic adaptation. Evolution 10: 105-108.

Martin J, Avery RA. 1998. Effects of tail loss on the movement patterns of the lizard, *Psammodromus algirus*. Funct Ecol 12: 794-802.

Meiri S, Dayan T. 2003. On the validity of Bergmann's rule. J Biogeogr 30: 331-351.

Meiri S, Dayan T, Simberloff D. 2005. Biogeographical patterns in the Western Palearctic: the fasting-endurance hypothesis and the status of Murphy's rule. J Biogeogr 32: 369-375.

Michael ED. 1972. Growth rates in *Anolis carolinensis*. Copeia 1972: 575-577.

Michaud EJ. 1990. Geographic variation of life history traits in the lizard *Anolis carolinensis*, Unpubl Ph.D. Diss. Univ. Tennessee, Knoxville.

Michaud EJ, Echternacht AC. 1995. Geographic variation in the life history of the lizard *Anolis carolinensis* and support for the pelvic constraint model. J Herpetol 29: 86-97.

Minesky JJ. 1999. Development and application of a genetic algorithm-informational modeling approach to exploratory statistical modeling of lizard-habitat relationships, Unpubl Ph.D. Diss, Univ. Tennessee, Knoxville.

Mongold JA, Lenski RE. 1996. Experimental rejection of a nonadaptive explanation for increased cell size in *Escherichia coli*. J Bacteriol 178: 5333-5334.

Oliver, JA. 1950. *Anolis sagrei* in Florida. Copeia 1950: 55-56.

Packard GC, Packard MJ. 1988. The physiological ecology of reptilian eggs and embryos. In: Biology of the Reptilia, Vol. 16, p. 525-605. Gans C, Huey RB, Eds. New York, Alan R. Liss, Inc.

Power M, McKinley RS. 1997. Latitudinal variation in lake sturgeon size as related to the thermal opportunity for growth. Trans Amer Fish Soc 126: 549-558.

Searcy WA. 1980. Optimum body sizes at different ambient temperatures: an energetics explanation of Bergmann's rule. J Theor Biol 83: 579-593.

Schoener TW. 1970. Size patterns in West Indian *Anolis* lizards. II. Correlations with the sizes of particular sympatric species- displacement and convergence. Amer Nat 104: 155-173.

Shine R. 2004. Adaptive consequences of phenotypic plasticity. In: Reptilian incubation: environment, evolution, and behaviour, p. 187-210. Deeming DC, Ed. UK, Nottingham

Univ. Press.

Sinervo B. 1990. Evolution of thermal physiology and growth rate between populations of the western fence lizard (*Sceloporus occidentalis*). Oecologia 83: 228-237.

Szarski H. 1983. Cell size and the concept of wasteful and frugal evolutionary strategies. J Theor Biol 105: 201-209.

Szarski H. 1985. Cell size in various vertebrate tissues. Fortschritte Der Zoologie 30: 313-316

Starostova Z, Kratochvil L, Frynta D. 2005. Dwarf and giant geckos from the cellular perspective: the bigger the animal, the bigger its erythrocytes? Funct Ecol 19: 744-749.

Stillwell RC, Morse GE, Fox CW. 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. Am Nat 170: 358-369.

Van der Have TM, De Jong G. 1996. Adult size in ectotherms: Temperature effects on growth and differentiation. J Theor Biol 183: 329-340.

Van Voorhies WA. 1996. Bergmann size clines: a simple explanation for their occurrence in ectotherms. Evolution 50: 1259-1264.

Warner DA, Andrews RM. 2002. Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. Biol J Linn Soc 76: 105-124.

Webb, SD. 1990. Historical Biogeography. In: Ecosystems of Florida. Myers RL, Ewel JJ, Eds., Orlando, Univ. Central Florida Press, pp. 70-100.

West GB, Woodruff WH, Brown JH. 2002. Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. PNAS 99: 2473-2478.

Yom-Tov Y, Geffen E. 2006. Geographic variation in body size: the effects of ambient temperature and precipitation. Oecologia 148: 213-218.

Appendix IV:

Tables and Figures

Table IV-1. 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.

Site	Dates		Station: Temps	Station: Precip
NC	1987-2007	35.472º N, 76.909º W	72309593719 NEW BERN CRAVEN CO REGL AP (35.068º N, 77.047º W; 47 km from site)	Same station as for Temps
E_TN	1986-2006	35.553º N, 84.077º W	72326013891 KNOXVILLE MCGHEE TYSON AP (35.818º N, 83.986º W; 31 km from site)	Same station as for Temps
W_TN	1987-2007	35.058º N, 88.190º W	72323513896 MUSCLE SHOALS REGIONAL AP (34.745º N, 87.610º W; 63 km from site)	Same station as for Temps
S_LA	1986-2006	30.404º N, 91.558º W	72240513976 LAFAYETTE REGIONAL AP (30.205 ºN, 91.988 ºW; 47 km from site)	Same station as for Temps
N_LA	1986-2006	32.455º N, 91.977º W	72248613942 MONROE REGIONAL AP (32.511º N, 92.038º W; 9 km from site)	Same station as for Temps
NE_FL	1987-2007	30.266º N, 81.512º W	72206593837 JACKSONVILLE NAS (30.233º N, 81.683º W; 17 km from site)	Same station as for Temps
NW_FL	1987-1998	30.373º N, 85.560º W	72224503882 PANAMA CITY BAY CO (30.200º N, 85.683º W; 23 km from site)	74777003852 VALPARAISO HURLBURT (30.429º N, 86.689º W; 108 km from site)
	1999-2007		" "	72224503882 PANAMA CITY BAY CO
SE_FL	1987-2007	26.844º N, 80.354º W	72203012844 WEST PALM BEACH INTL ARPT (26.685º N, 80.099º W; 31 km from site)	Same station as for Temps
SW_FL	1987-2007	25.993º N, 81.599º W	72210612835 FORT MYERS PAGE FIELD (26.586º N, 81.864º W; 71 km from site)	Same station as for Temps
M_FL	1987-2007	28.632º N, 81.125º W	72205012815 ORLANDO INTL ARPT (28.434º N, 81.325º W; 29 km from site)	Same station as for Temps
OR_TX	1987-2007	30.127º N, 93.704º W	72241012917 PORT ARTHUR JEFFERSON CO (29.951º N, 94.021º W; 36 km from site)	Same station as for Temps

Table IV-1. Continued

Site	Dates		Station: Temps	Station: Precip
AL	1987-2007	32.549º N, 85.311º W	72225593842 COLUMBUS METROPOLITAN ARPT (32.516º N, 84.942º W; 35 km from site)	Same station as for Temps
TY_TX	1986-1989	32.256º N, 95.184º W	72244813972 TYLER POUNDS FIELD (32.354º N 95.402º W; 23 km from site)	72258113960 DALLAS LOVE FIELD (32.847º N, 96.851º W; 33 km from site)
	1990-1992		" "	72258313960 DALLAS LOVE FIELD (same station as above)
	1993-1999		72258313960 DALLAS LOVE FIELD	" "
	2000		72244813972 TYLER POUNDS FIELD	" "
	2001-2006		" "	72244813972 TYLER POUNDS FIELD
CC_TX	1986-2006	27.682º N, 97.330º W	72251512926 CORPUS CHRISTI NAS (27.700º N, 97.283º W; 5 km from site)	Same station as for Temps
BV_TX	1987-2007	25.997º N, 97.567º W	72250012919 BROWNSVILLE S PADRE ISL INTL A (25.906º N, 97.426º W; 17 km from site)	Same station as for Temps
MS	1987-01/1999	30.592º N, 88.621º W	72223013894 MOBILE REGIONAL AP (30.688º N, 88.246º W; 37 km from site)	Same station as for Temps
	02/1999-03/2005		74768899999 PASCAGOULA (30.464º N, 88.532º W; 17 km from site)	Same station as for Temps
	04/2005-2007		74768853858 PASCAGOULA (same station as above)	Same station as for Temps
GA	1986-2006	33.477º N, 81.983º W	GA 72218003820 AUGUSTA BUSH FIELD (33.467º N, 82.033º W; 5 km from site)	Same station as for Temps
AR	1987-2006	34.230º N, 93.134º W	72341599999 MEMORIAL FLD (34.467º N, 93.083º W; 27 km from site)	Same station as for Temps

Table IV-1. Continued

Site	Dates		Station: Temps	Station: Precip
SC	1987-1990	33.651º N, 78.931º W	74791593718 NORTH MYRTLE BEACH GRAND STRA (33.816º N, 78.721º W; 27 km from site)	72310613744 FLORENCE REGIONAL AP (34.188º N, 79.731º W; 95 km from site)
	1991		data incomplete; not used	" "
	1992-1996		74791593718 NORTH MYRTLE BEACH GRAND STRA	" "
	1997-1999		" "	data incomplete; not used
	2000-2004		74791099999 MYRTLE BEACH CIV (33.683º N, 78.933º W; 4 km from site)	72310613744 FLORENCE REGIONAL AP
	2005-2007		74791593718 NORTH MYRTLE BEACH GRAND STRA	" "

Table IV-2. Sample size,average, standard deviation, and range of snout-vent length (SVL) for male and female *Anolis carolinensis* in 19 populations.

	Females				Males			
Population	**N**	**Average**	**SD**	**Range**	**N**	**Average**	**SD**	**Range**
NW_FL	17	50.7	1.5	48.0 – 53.0	16	60.0	1.8	55.0 – 63.0
NE_FL	16	50.8	3.7	40.0 – 55.5	17	60.6	5.2	48.0 – 66.5
M_FL	16	45.9	1.8	43.5 – 50.0	17	51.5	2.9	44.5 – 56.0
SW_FL	1	-	-	45.5	16	55.7	2.1	52.0 – 59.0
SE_FL	15	44.5	1.9	42.0 – 49.5	17	51.9	3.0	46.0 – 57.0
AR	21	53.9	2.9	48.5 – 59.0	19	57.7	5.5	49.5 – 67.0
E_TN	21	51.4	2.6	48.0 – 57.0	21	59.9	4.2	50.0 – 66.0
W_TN	18	53.1	2.9	48.0 – 55.5	15	57.9	5.4	45.0 – 65.0
TY_TX	20	55.2	1.6	53.0 – 58.5	19	62.3	4.4	51.0 – 68.0
OR_TX	16	58.0	2.0	56.0 – 62.0	16	70.5	2.8	63.0 – 74.0
CC_TX	18	56.1	2.6	49.0 – 60.0	19	64.4	5.0	54.0 – 69.5
BV_TX	2	-	-	54.0 – 55.5	8	66.9	3.4	60.0 – 71.0
AL	16	53.3	3.0	49.0 – 60.0	18	56.6	4.9	45.0 – 63.0
SC	15	54.2	5.0	49.0 – 69.0	14	61.3	5.3	52.0 – 69.0
NC	18	51.9	4.3	43.0 – 60.0	15	58.3	5.8	48.0 – 66.5
GA	17	54.1	1.7	51.0 – 57.5	18	62.3	4.7	52.5 – 71.0
S_LA	21	54.7	2.4	47.0 – 58.0	21	67.5	4.9	52.5 – 73.0
N_LA	20	55.6	2.5	52.0 – 60.5	21	65.0	4.8	54.0 – 71.5
MS	16	56.6	2.4	50.5 – 60.0	16	67.5	3.7	59.0 – 72.0

Table IV-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. Sample sizes for RBC and MC are shown below each population. R-squared and statistics for t tests of slope estimates are shown. Bolded p-values denote significance after Bonferroni correction for multiple tests (corrected alpha = 0.00088).

Population	**Y**	**r^2**	**Slope ± SE**	**t**	**df**	**p**
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
	MC size	0.79	97.589 ± 8.355	11.68	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**
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**
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**
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**
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**
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**
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**
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**
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**
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**

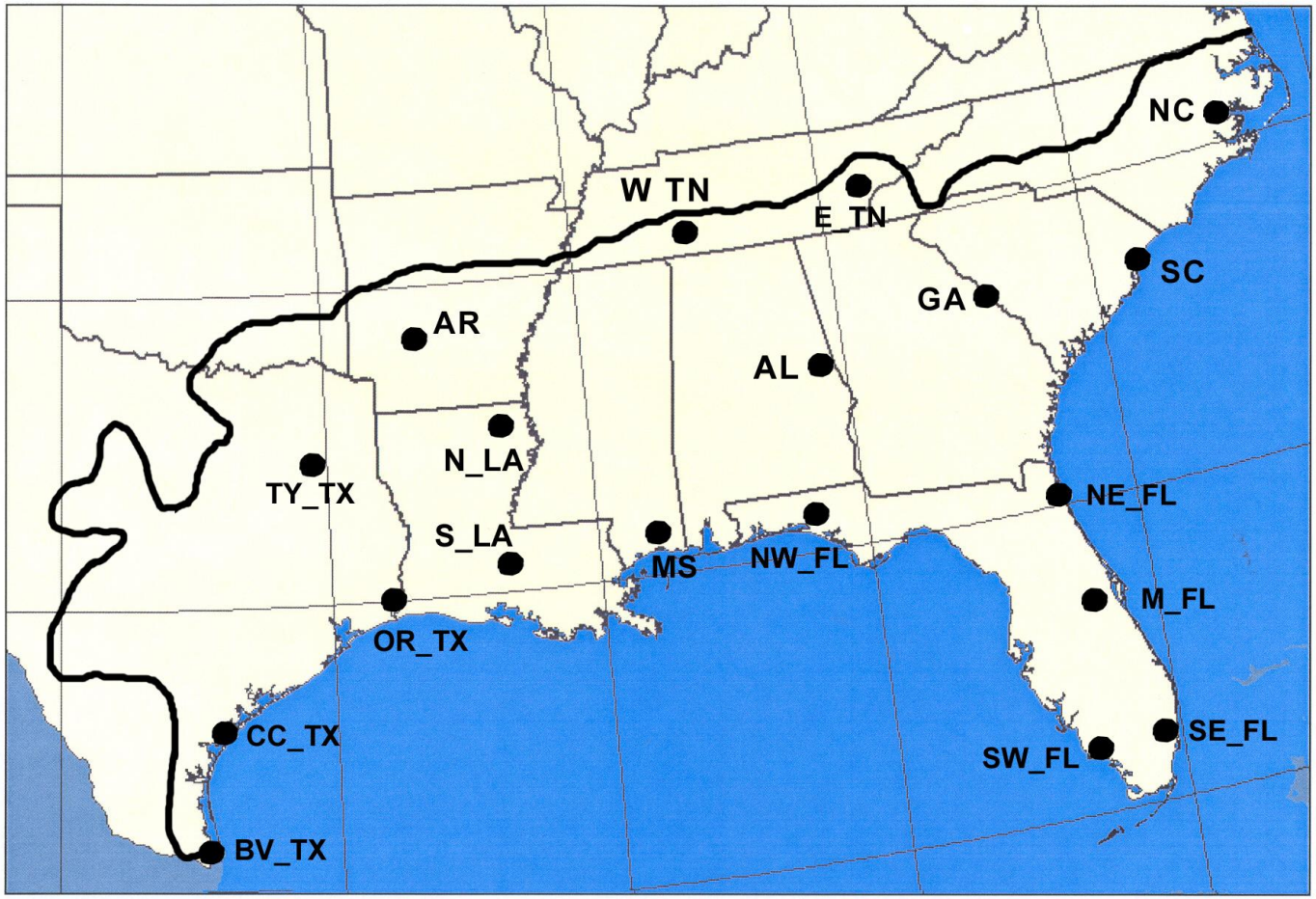

Fig IV-1. Range map of *Anolis carolinensis*, showing locations of 19 populations sampled in the current study. The bolded line shows the outer limit of the species range.

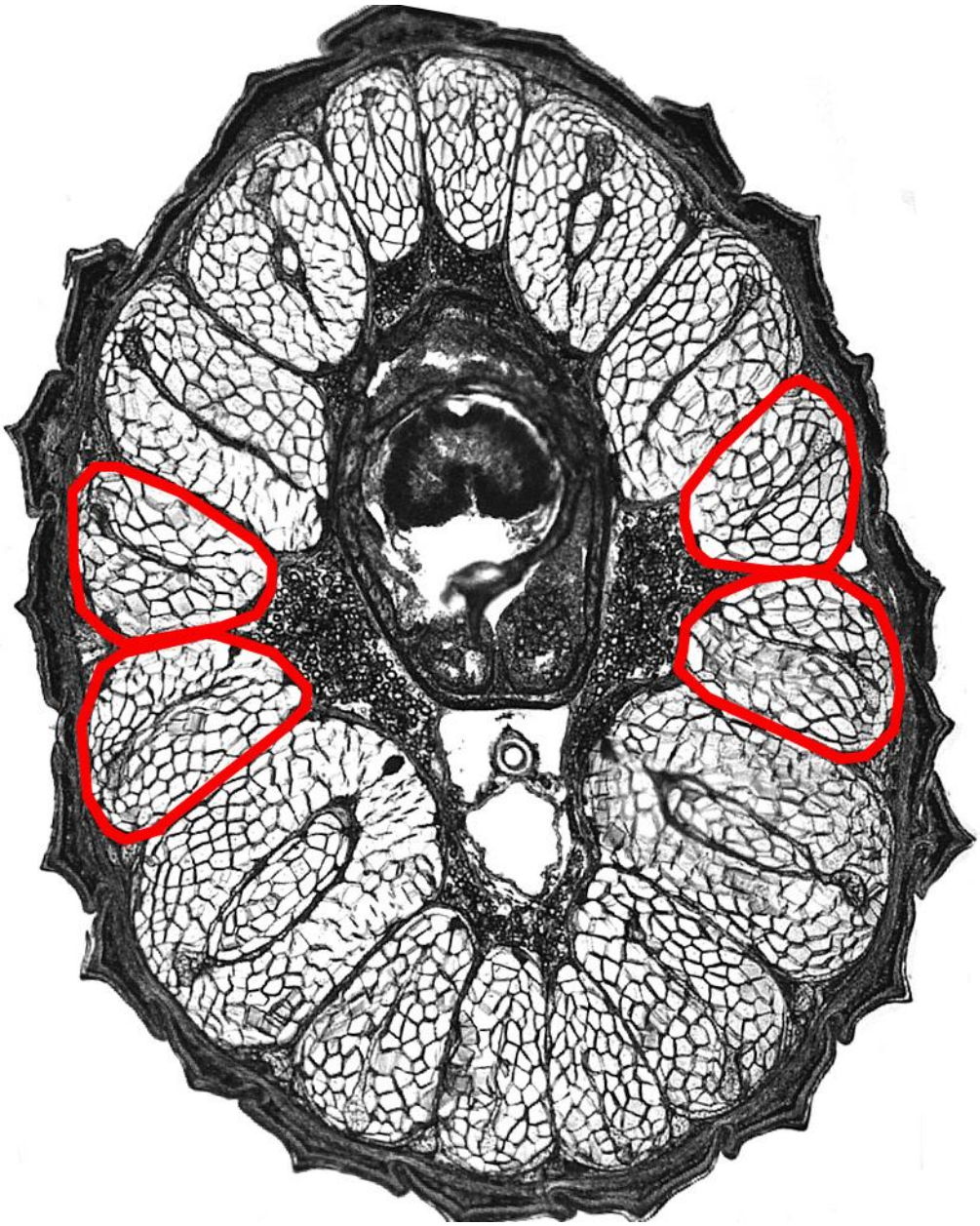

Figure IV-2. Transverse section through tail of *Anolis carolinensis*, indicating the four muscles (circled in red) sampled in the current study.

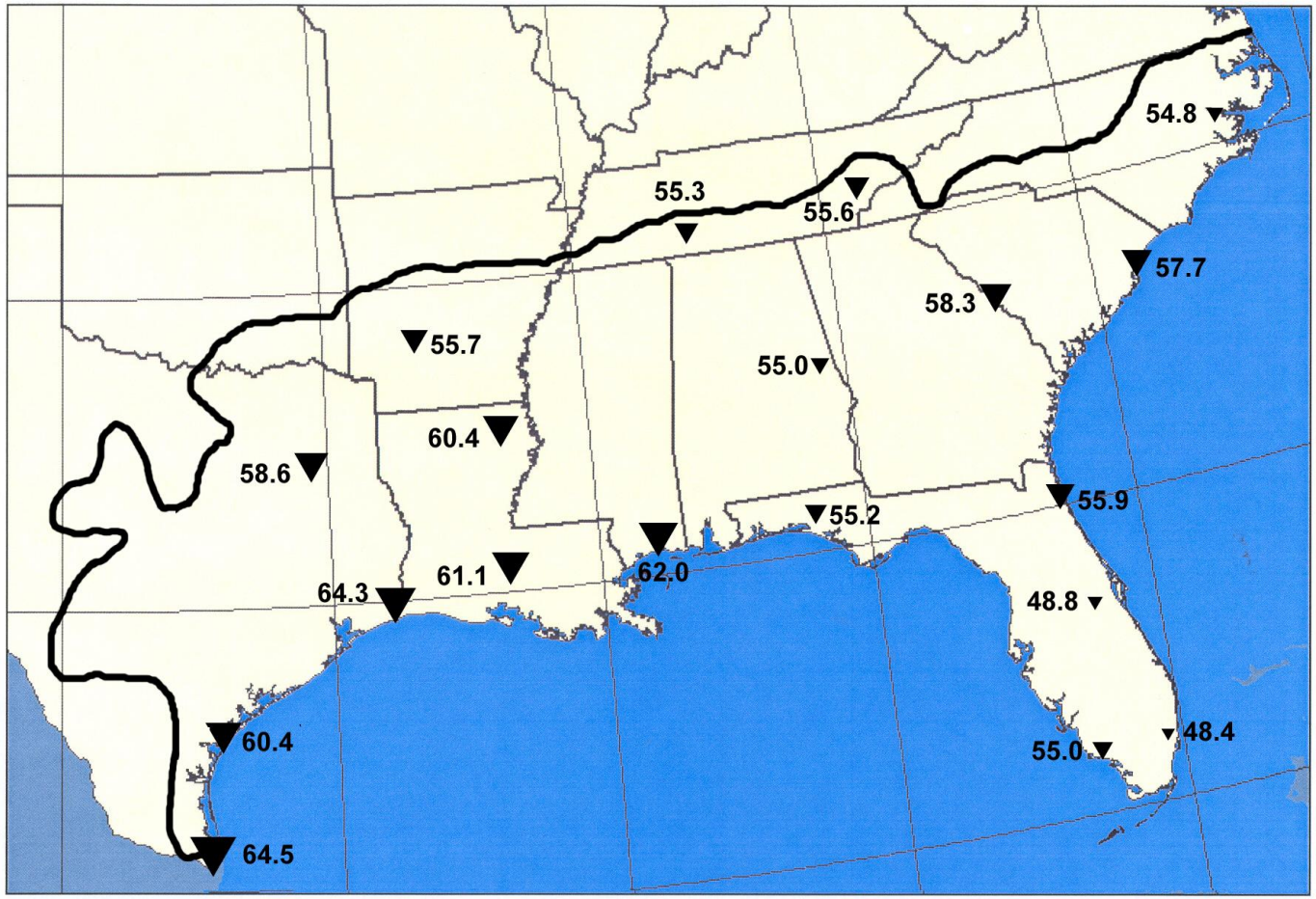

Fig IV-3. Average snout vent length (cm) of *Anolis carolinensis* in 19 populations. The bolded line shows the outer limit of the species range.

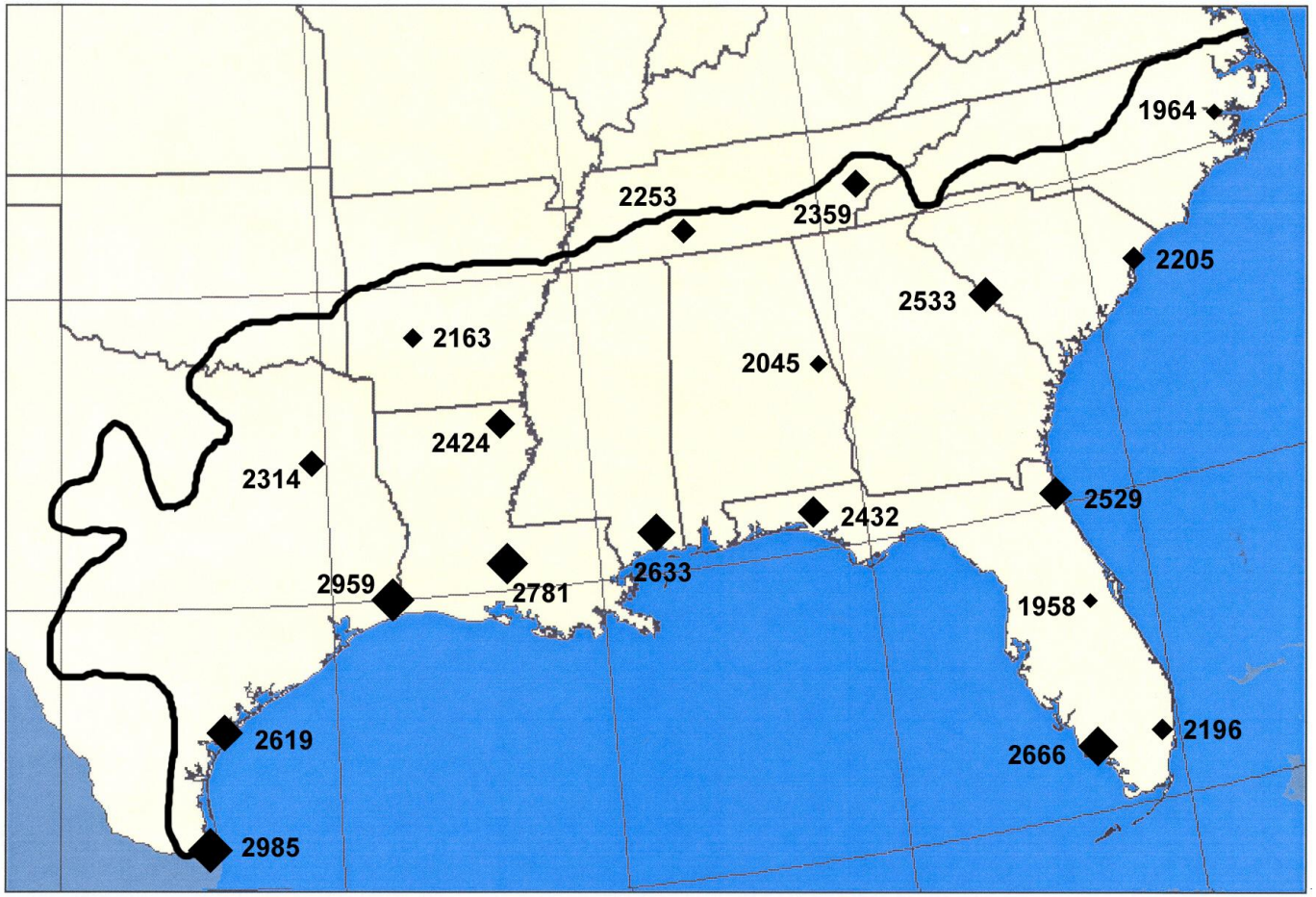

Fig IV-4. Average muscle cell size (μm^2) of *Anolis carolinensis* in 19 populations. The bolded line shows the outer limit of the species range.

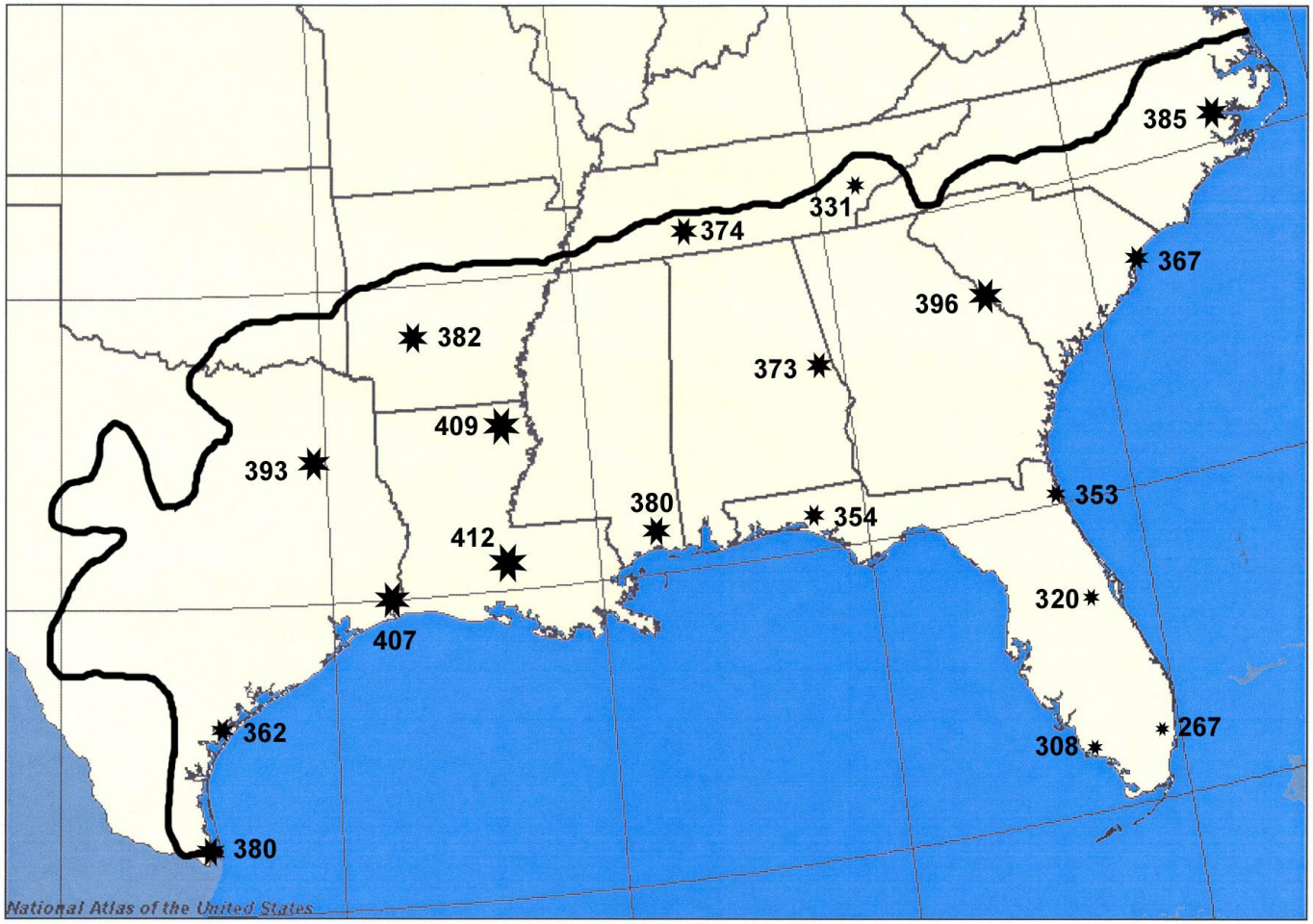

Fig IV-5. Average muscle cell number in four segments of skeletal muscle (viewed in cross-section) from tails of *Anolis carolinensis* from 19 populations. The bolded line shows the outer limit of the species range.

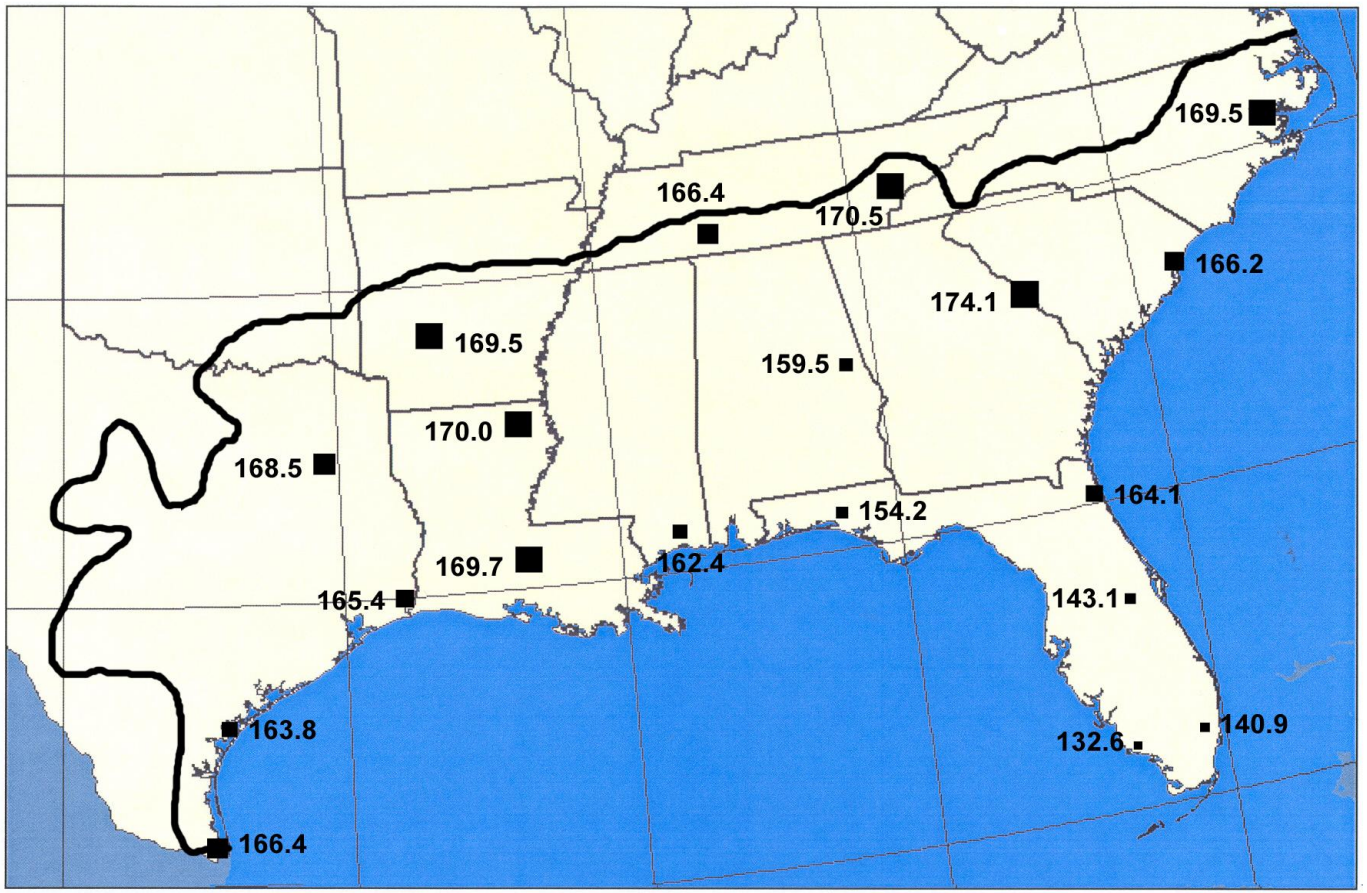

Fig IV-6. Average red blood cell size (μm^2) of *Anolis carolinensis* in 19 populations. The bolded line shows the outer limit of the species range.

CONCLUSIONS

This dissertation illustrates how environmental temperature and other factors related to geographic distribution shape the morphology of the lizard *Anolis carolinensis* on both developmental and evolutionary time scales. The main findings of the four parts of the dissertation are summarized below.

Part I: Eggs and juveniles from four populations in TN, GA, and FL were reared under identical conditions to determine whether differences in body size were attributable to maternal investment in egg size and/or to growth rates of embryos and juveniles. To control for differences in maternal investment in egg size among populations, yolk was removed from eggs in two northern populations to produce eggs comparable in size to those of one southern population. This study confirmed previously suggested trends of larger adult size and egg size in the north. Additionally, I found that differences in juvenile and, potentially, embryonic growth rates existed among populations of *A. carolinensis*, both due to and independent of differences in egg size. Juveniles from the northernmost population were bigger not only due to larger egg size, but also due to faster juvenile growth and possibly difference in developmental stage of oviposition or conversion of egg mass to hatchling mass.

Part II: Eggs from four populations in TN, GA, and FL were incubated at up to three temperatures, 23.5, 27, and 30 °C. Measures of body size were collected at hatching and for eight weeks after, while juveniles were maintained in a common laboratory environment. Cooler incubation temperatures resulted in longer incubation periods but did not affect conversion of egg mass to hatchling mass. Incubation temperature did not affect hatchling body size, but did affect subsequent growth rates, which also varied by population. Cooler incubation temperatures

generally resulted in greater overall growth over eight weeks during which all juveniles were housed in a common environment. In *A. carolinensis,* egg incubation temperature had latent effects on juvenile growth despite the absence of effects on initial hatchling size.

Part III: Predictions of the temperature size rule were tested for *A. carolinensis* within the experiments described above. This rule describes a widespread pattern wherein ectotherms reared in cooler temperatures experience slowed growth and developmental rates, but reach a larger size than those from warmer temperatures. Expanding on this, research has suggested that the pattern of growing larger in cooler environments may also apply at the cellular level in ectotherms. This study 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 different temperatures. Larger cells were produced in hatchlings from cooler treatments; however, initial hatchling body size was unaffected. Therefore, temperature-induced plasticity does not apply consistently at cellular and organismal levels in *A. carolinensis.*

Part IV: Body size and sizes of erythrocytes and skeletal muscle cells were estimated for adults of both sexes in 19 populations of *A. carolinensis* across the entire species range. This observational study showed a longitudinal pattern in body size, driven by small body size in Florida populations. If the latter were excluded, a trend became evident with larger body size in southern latitudes. This negative association between body size and latitude mirrors the general pattern in squamates. Both types of cells were larger in western populations, but again, these trends were driven entirely by small cell size in Florida. Exclusion of those five populations nullified any longitudinal trends in cell size. Environmental temperatures decrease and seasonality increases with latitude in the range of *A. carolinensis*. Theory suggests that northern

environments would produce larger cell size for greater metabolic efficiency. However, this prediction was not met in the current study, wherein muscle cell size was negatively related to latitude, and red blood cell size showed no latitudinal trend outside of Florida. The different patterns in the two cell types confirm the importance of examining multiple cell types when studying geographic variation in cell size.

VITA

Rachel Madeline Goodman was born in Storm Lake, Iowa on June 19, 1980. She lived with her family in Iowa until 1984, then in Colombia, South America from 1985-1987. She completed the majority of her primary and secondary education in El Paso, Texas, and graduated from Socorro High School in 1997. She then attended Columbia University and received her Bachelor of Arts degree in Environmental Biology in May of 2001. During this time, Rachel studied and did research in Spain, Costa Rica, New York state, and the Biosphere 2 in Arizona. She joined the Department of Ecology and Evolutionary Biology at the University of Tennessee, Knoxville in August of 2001 and received her Master of Science degree in May of 2004. During this program, she studied the behavior and conservation of the endangered iguana *Cyclura lewisi* on Grand Cayman. Rachel remained at the University of Tennessee, Knoxville and began her doctoral studies and the research described herein in the fall of 2004. After receiving her Ph.D. in the summer of 2009, she will become an Assistant Professor in the Department of Biology at Hampden-Sydney College in Virginia.