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CONVERGENT EVOLUTION OF EMBRYONIC GROWTH AND DEVELOPMENT IN THE EASTERN FENCE LIZARD (*SCELOPORUS UNDULATUS*)

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Abstract.—Theory predicts that cold environments will select for strategies that enhance the growth of ectotherms, such as early emergence from nests and more efficient use of resources. We used a common garden experiment to detect parallel clines in rates of embryonic growth and development by eastern fence lizards (*Sceloporus undulatus*). Using realistic thermal conditions, we measured growth efficiencies and incubation periods of lizards from five populations representing two distinct clades. In both clades, embryos from cold environments (Indiana, New Jersey, and Virginia) grew more efficiently and hatched earlier than embryos from warm environments (Florida and South Carolina). Because eggs from cold environments were larger than eggs from warm environments, we experimentally miniaturized eggs from one population (Virginia) to determine whether rapid growth and development were caused by a greater maternal investment. Embryos in miniaturized eggs grew as efficiently and incubated for the same duration as embryos in unmanipulated eggs. Taken together, our results suggest countergradient variation has evolved at least twice in *S. undulatus*.

Key words.—Convergent evolution, countergradient variation, development, growth efficiency, *Sceloporus undulatus*.

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Geographically widespread ectotherms encounter thermal gradients that generate variation in behavior, physiology, and life history (Dunham et al. 1989; Porter 1989; Angilletta et al. 2002). Daily and seasonal patterns of environmental temperature affect profiles of body temperatures in both thermoregulators and thermoconformers. Consequently, thermal environments determine the duration and effectiveness of foraging (Grant and Dunham 1990; Ayers and Shine 1997; Wapstra and Swain 2001), consumption and assimilation (Ji et al. 1995; Kingsolver and Woods 1997; Angilletta 2001). These thermal constraints on behavioral and physiological processes generate latitudinal and altitudinal variation in the growth, development, and maturation of ectotherms (Beaupre 1995; Niewiarowski 2001; Sears and Angilletta 2004).

Because rates of physiological processes are very sensitive to temperature, cold environments are thought to favor genotypes that have relatively high capacities for growth despite energetic constraints (Sibly and Calow 1983; Taylor and Williams 1984). Three different adaptive responses can occur: (1) mothers in colder environments can allocate more energy to each offspring, (2) offspring in colder environments can make more efficient use of available resources, or (3) offspring in colder environments can emerge earlier to compensate for a shorter growing season (Fig. 1). The first strategy appears to be common among ectotherms because females in colder environments tend to produce larger eggs (Perrin 1988; Iguchi and Yamaguchi 1994; Glazier 1999; Ernsting and Isaaka 2000; Tamate and Maekawa 2000; Kim and Thorpe 2001; reviewed by Atkinson et al. 2001). Moreover, selection experiments involving *Drosophila melanogaster* have been used to link the evolution of large eggs directly to low environmental temperature (Azevedo et al. 1996). Larger eggs presumably contain more energetic resources for offspring and result in a larger size at hatching (Sinervo 1990) or a larger reserve of lipids after hatching (Congdon 1989). Evidence of thermal adaptation of embryonic growth and development is less abundant, but adaptive strategies of growth have become increasingly apparent from studies of other life stages (see reviews by Arendt 1997; Gotthard 2001; Sears and Angilletta 2004). Growth in colder environments can be enhanced by modifying rates of consumption, digestion, and respiration (Lonsdale and Levinton 1989; Ayres and Scribner 1994; Billerbeck et al. 2000; Sears and Angilletta 2004). However, warm environments might select against rapid growth, if it imposes a cost, such as a greater risk of predation with increased feeding (Billerbeck et al. 2000; Gotthard 2000). Thus, natural selection can produce genetically induced variation in growth and development that counteracts thermally induced variation (Conover and Schultz 1995). Still, the relative effects of maternal and embryonic strategies on rates of growth and development are largely unknown.

We report evidence that the rates of embryonic growth and development have evolved in parallel between two clades of the eastern fence lizard (*Sceloporus undulatus*). In northern populations, females of *S. undulatus* produce relatively large eggs from which emerge relatively large hatchlings (Angilletta et al. 2005). One possibility is that the larger body size of northern hatchlings is due entirely to the maternal effect of egg size (Bernardo 1996). Still, much of the variation in hatchling size cannot be accounted for by variation in egg mass, and some of this residual variation in hatchling size could be caused by adaptive strategies of embryonic growth and development. We predicted that embryos from northern populations would have evolved capacities to grow more efficiently and develop more rapidly than embryos from southern populations because of differences in their thermal environments. To test this prediction, we incubated embryos from five populations in two common environments, and compared their growth efficiencies and incubation periods.

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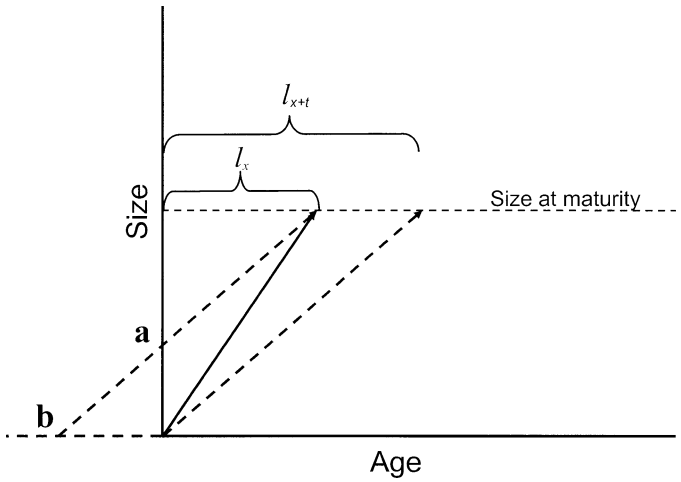

FIG. 1. A conceptual model of strategies that enhance the growth of juveniles in cold environments. Dashed lines represent growth in cold environments, whereas the solid line represents growth in warm environments. Assuming a constant survivorship of juveniles and a minimal size at maturity, individuals in cold environments are less likely to survive to maturity than individuals in warm environments. ($l_{x+t} < l_x$). In colder environments, natural selection is thought to produce the evolution of certain strategies: (a) a larger size at hatching, which could result from a greater allocation of energy by mothers or more efficient use of energy by embryos, and (b) a shorter period of embryonic development to provide more time for growth as a juvenile.

Furthermore, we experimentally reduced the sizes of eggs from a northern population to disentangle the effects of energy availability and population of origin on embryonic growth and development.

METHODS

Sampling of Populations

We used a recent phylogeographic analysis of *Sceloporus undulatus* (Fig. 2, Leaché and Reeder 2002; see also Angilletta et al. 2004) to select populations from two clades that are separated by the Appalachian Mountains of the eastern United States. East of the Appalachians (eastern clade), we sampled gravid females from Burlington County, New Jersey (NJ) and Aiken County, South Carolina (SC). West of the Appalachians (western clade), we sampled gravid females from Monroe County, Indiana (IN) and Santa Rosa County, Florida (FL). Mean annual air temperatures in NJ and IN (12.4° and 11.9°C, respectively) are much lower than those in SC and FL (18.0° and 19.2°C, respectively). Additionally, we sampled a population from a relatively high altitude (600 m) in Montgomery County, Virginia (VA), because its environmental temperature (11.0°C) was similar to those of the northern populations. Thus, we expected the growth efficiencies and incubation periods of embryos from NJ, VA, and IN to differ from those of embryos from SC and FL.

Collection and Care of Eggs

In 2002 and 2003, we obtained eggs from gravid females collected in each of the populations. Females were housed in terraria (38 L), which were placed in an environmentally controlled room. The photoperiod was 12L:12D and the ambient temperature was 23°C. An incandescent bulb was placed on one side of each cage to enable behavioral thermoregulation. Food (domesticated crickets) and water were available ad libitum. Females were maintained under these conditions from the time they were brought into the laboratory until the time they were released.

To accurately determine the energy contents of eggs from their initial masses, we needed to weigh eggs as soon as they were laid. Otherwise, eggs would have exchanged water with their environment, which would have weakened the relationship between egg mass and energy content. By hormonally inducing females to oviposit, we were able to collect eggs and weigh them before significant exchanges of water occurred. Approximately two days after entering the laboratory, we injected each female with 0.3–0.5 ml of oxytocin (6–10 USP units; The Butler Company, Columbus, OH). Following the injection, females were placed in ventilated plastic containers where they could be observed throughout the day. These containers were held in an illuminated incubator set at 30°C (Precision Model 818, Precision Scientific, Chicago, IL). This procedure enabled us to observe and collect eggs, while minimizing stress to the females. Most females laid their eggs within five hours of receiving the injection. During this period, eggs of each clutch were collected and weighed to the nearest 0.1 mg.

Experimental Design

Because clutch size varied considerably among females (range = 6–14 eggs), a stratified random sampling design was used to assign eggs to each treatment. First, two eggs from each clutch were stored in gaseous nitrogen at −80°C, enabling us to estimate the energetic content of eggs at oviposition (see below). Then, two eggs were randomly assigned to each of two treatments during incubation: a warm cycle that ranged from 20° to 34°C with a mean of 27°C, and a cool cycle that ranged from 20° to 30°C with a mean of 24°C (Fig. 3). These thermal cycles were based on nests constructed by females in artificial thermal gradients and natural environments (Warner and Andrews 2002; M. Angilletta, R. Pringle, and M. Sears, unpubl. data). Thermal cycles were created with two programmable incubators (Model KB 115, Brinkmann Instruments, Westbury, NY) controlled by commercially available software (APT-COM, Binder, Tuttlingen, Germany).

Except for temperature, the environments during incubation were similar for all eggs. Eggs were placed in plastic containers (0.5 L) filled with approximately 450 g of fine sand and 4.5 g of distilled water. Two eggs were incubated in each container; pairs of eggs were never from the same population to avoid systematic covariation between the incubation environment and the population of origin (hereafter referred to as ''population''). Based on the tensiometry, the water potential during incubation was −10 kPa. To maintain this water potential, we weighed the containers every few days and replaced evaporated water with a syringe. At these times, the positions of the containers within the incubators were rotated to avoid spatial effects.

The growth and development of embryos was assessed

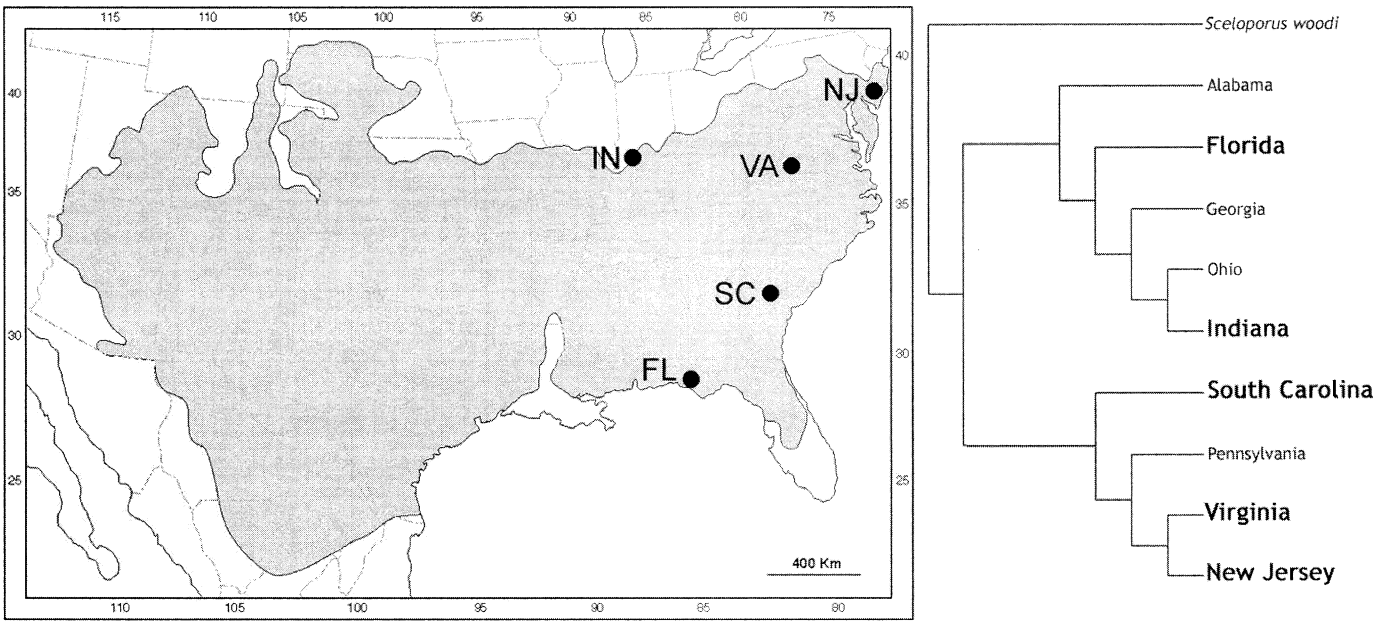

FIG. 2. A map showing the locations of the five populations sampled for our study of embryonic physiology. The phylogenetic relationships among these populations are shown to the right of the map. The cladogram is redrawn from Angilletta et al. (2005).

through several measures. After the first few weeks of incubation, containers were checked daily to determine dates of hatching. Incubation period was calculated as the number of days between oviposition and hatching. The mass and length of each hatchling was also measured. Hatchlings were sacrificed and carcasses were stored in gaseous nitrogen at −80°C until their compositions and caloric densities could be measured; these energetic measures enabled us to calculate growth efficiencies (see Calculation of Growth Efficiency).

When comparing growth and development between populations of embryos, one should be concerned about a bias in the developmental stage at oviposition (Qualls and Shine 1998). Embryos at later stages should hatch earlier and would appear to grow more efficiently than embryos at earlier stages. Data for species of *Sceloporus* confirm that developmental stage at oviposition varies among clutches and correlates with an embryo's incubation period (Sexton and Marion 1974; DeMarco 1993). In our experiment, developmental stage at oviposition probably varied within populations and possibly varied among populations. But available data indicate variation among populations was likely neither significant nor systematic. In a recent study of *S. undulatus* (Parker et al. 2004), embryos of females induced hormonally were no less developed than embryos of females that oviposited naturally; moreover, developmental stages at oviposition did not differ between females from NJ and those from SC—two of the populations included in our experiment. Similarly, Qualls and Shine (1998) observed very little variation in embryonic stage within and between populations of scincid lizards (*Lampropholis guichenoti*). Based on these data, we believe our measures of growth efficiencies and incubation periods enabled us to test hypotheses about embryonic growth and development.

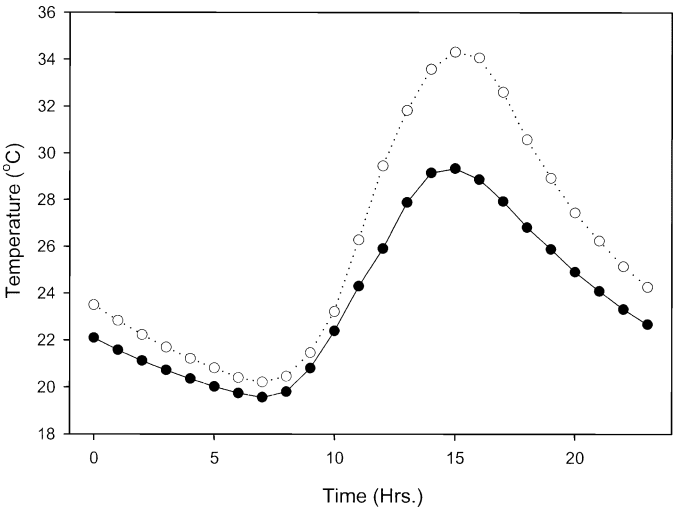

FIG. 3. Two thermal cycles, with mean temperatures of 24° and 27°C, were used during the incubation of eggs. These thermal cycles mimic those of natural nests in Virginia and New Jersey (Warner and Andrews 2002; M. Angilletta, R. Pringle, and M. Sears, unpubl. ms.).

Yolk Removal

Intraspecific variation in embryonic growth and development can be caused not only by genetic divergence but also by maternal allocation (Sinervo and McEdward 1988). Because eggs vary considerably in size among populations of *S. undulatus* (Niewiarowski et al. 2004), energy availability is a confounding maternal effect in our comparisons of embryonic physiology. To control for the effect of energy availability, we experimentally reduced the energy content of a subset of eggs from VA. Reduction of yolk produces variation in the body size of hatchlings that mimics that caused by natural variation in egg size (Sinervo 1990; Warner and An-

TABLE 1. Regression models of the relationship between the wet mass of an egg (g) and its energy content (kJ) for five populations of *Sceloporus undulatus*. The coefficient (β) and intercept (α) of each model are provided as well as the median % deviation between predicted and actual values of energy content. The number of eggs (*n*), mean egg mass, and mean energy content (± 95% confidence intervals) are reported for each population. The differences among model parameters for the five populations reflect not only variation in the water contents of eggs within and among populations, but also variation in the composition of macromolecules in eggs within and among populations (C. Oufiero, A. Smith, and M. Angilletta, unpubl. data).

Population	*n*	Egg mass (mg)	Energy content (kJ)	β	α	r^2	Median % deviation
Indiana	49	409 ± 19	3.85 ± 0.15	3.88	2.26	0.22	7.1%
Florida	23	317 ± 37	3.40 ± 0.21	4.22	2.06	0.57	6.9%
New Jersey	32	384 ± 23	4.14 ± 0.12	2.28	3.27	0.21	4.4%
Virginia	37	440 ± 21	4.22 ± 0.15	6.00	1.58	0.68	4.1%
South Carolina	23	351 ± 12	3.88 ± 0.12	1.34	3.40	0.02	5.1%

drews 2003). Yolk was removed from 15 and 20 eggs in 2002 and 2003, respectively. A 26.5 gauge syringe was inserted in the yolk sac of each egg and a portion of yolk was aspirated (85.3 ± 10.4 mg or 18 ± 1% of total egg mass [mean and 95% confidence interval]). After this procedure, these eggs were randomly assigned to one of the thermal treatments and were incubated with the unmanipulated eggs.

To calculate growth efficiency, we needed to estimate the caloric content of the yolk that was removed from each egg. Retrieving all of the yolk from each syringe was impossible, but a sufficient sample could be obtained for most eggs. These samples were stored in gaseous nitrogen at −80°C until we could measure their caloric densities. Later, the samples were lyophilized and weighed to the nearest 0.1 mg. The caloric density of each sample was measured with a bomb calorimeter (Model 1425, Parr Instrument Co., Moline, IL), which was calibrated daily using benzoic acid as a standard. The dry mass of yolk removed from each egg was estimated from the change in egg mass and the mean water content of yolk (50.8 ± 8.7%). Finally, we multiplied this dry mass by the mean caloric density of yolk to yield the calories extracted during yolk reduction.

Measuring Caloric Contents of Eggs

We constructed models describing the relationship between the wet mass of an egg at oviposition and its caloric content. Eggs that were frozen at oviposition were lyophilized for 24 hours, weighed to the nearest 0.1mg, and homogenized with a mortar and pestle. Homogenized samples were compressed into pellets (≈50 mg each), which were combusted in a bomb calorimeter. The energetic densities of pellets were used to calculate the caloric contents of eggs. Linear regression was used to determine relationships between wet egg mass and caloric content. Parameters were estimated for each population separately because the composition of eggs might have differed among populations (e.g., see Booth 2003). Generally, these models predicted energy contents of eggs well within 10% of the actual values (Table 1). These models were used to estimate the energy available for growth during incubation (see Calculation of Growth Efficiency).

Measuring the Composition of Hatchlings

To determine the growth of embryos, we measured the percentages of hatchling body mass attributable to lean tissue and stored lipid. Hatchlings were lyophilized for 48 hours, weighed to the nearest 0.1 mg, and homogenized with a mortar and pestle. Each sample was then divided between two applications: half of the sample was combusted to obtain a caloric density of the hatchling, while the other half was reduced to lean tissue by extracting nonpolar lipids in a Soxhlet apparatus (for details of this procedure, see Angilletta 1999). Extracted samples were combusted to determine the caloric density of lean tissue. The caloric density of nonpolar lipid was calculated indirectly from measures of body composition and the caloric densities of extracted and unextracted tissues.

In 2002, an equipment malfunction prevented some hatchlings from lyophilizing completely. Therefore, we estimated dry masses of these hatchlings from a regression model of the relationship between wet mass and dry mass. This model was parameterized using data from 2003 ($\beta = 0.810$, $\alpha = 0.015$, Adjusted $r^2 = 0.66$, $P < 0.0001$, $n = 140$). Measures of caloric densities were unaffected by this problem because subsamples of tissue were dried completely before and after the extraction of lipids. Although this difference in procedure between 2002 and 2003 should not have introduced any systematic bias, we explored this possibility in our analysis of growth efficiency (see Statistical Analyses).

Calculation of Growth Efficiency

Measures of energetics were used to calculate the growth efficiency of each embryo. The net growth efficiency (K_2) of an embryo was defined as follows: $K_2 = G/A$, where G is the growth of lean tissue and A is the energy available for growth during incubation. This definition is consistent with traditional applications of the term net growth efficiency to embryonic growth (Wieser 1994). We did not consider nonpolar lipid to be growth because much of the lipid extracted from hatchlings is likely to be residual yolk absorbed at hatching (Troyer 1987). Nevertheless, our conclusions about growth efficiency would have been the same if nonpolar lipids were considered to be growth because they composed a small fraction of the caloric content of hatchlings (see Results). The growth of lean tissue was calculated by multiplying lean mass by its caloric density. The energy available for growth was calculated from regression models of the relationship between the wet mass of an egg and its energy content. By using this approach, we assumed that respiration by the embryo before oviposition consumed a negligible portion of energy available at the time of fertilization (A). Although this

assumption is not strictly valid, we estimated that violation of this assumption resulted in less than 3% error in our estimation of *A*, based on rates of embryonic respiration at oviposition (mean = 0.25 Jh^{-1}; C. Oufiero and M. Angilletta, unpubl. data) and durations of egg retention for closely related sceloporines (modal values are ≤16 days; DeMarco 1993). More likely, the actual error in *A* was less than 1% because (1) the rate of respiration used in our calculation was undoubtedly higher than actual rates from fertilization to oviposition, and (2) the duration of egg retention was less than that of females laying naturally.

Statistical Analyses

We used general linear models to analyze the effects of population and incubation temperature on incubation period, growth efficiency, and hatchling size (snout–vent length and mass). When possible, we used maternal identity (clutch) as a random factor to control for the dependence of traits among closely related individuals; when insufficient replication precluded this approach, we analyzed the mean values of each clutch to avoid inflating degrees of freedom through pseudoreplication (Potvin 2001). In our analysis of growth efficiency, the year of data collection was also included as a factor to determine if the variation between temperatures and populations was affected by the difference in methods between 2002 and 2003 (see Measuring the Composition of Hatchlings). Effects on growth efficiency were determined by ANCOVA, in which *G* was the dependent variable and *A* was the covariate; in this way, we avoided the direct analysis of percentages which commonly violate the assumptions of statistical models (Raubenheimer and Simpson 1994; Packard and Boardman 1999). Effects on hatchling size were also determined by ANCOVA, in which egg mass was used as a covariate. A model incorporating separate slopes was used when slopes of the relationship between the covariate and the independent variable differed among populations. All analyses were performed using Statistica 6.0 (StatSoft, Inc. 2003).

In each clade, we made planned comparisons of phenotypes between populations from cold and warm environments. For growth efficiency and size at hatching, we predicted IN > FL and VA ≥ NJ > SC. For incubation period, the opposite patterns were predicted. Because these alternative hypotheses were directional, we used the ordered heterogeneity test (OHT) of Rice and Gaines (1994a,b); this procedure converts the results of any two-sided test (e.g., ANOVA) into those of a one-sided test of a directional alternative hypothesis.

RESULTS

We observed indirect evidence of countergradient variation in developmental rate. Embryos from all populations incubated longer at the lower temperature (western clade: MS = 1674.1, $F_{1,39} = 986.6$, $P < 0.00001$; eastern clade: MS = 4602.7, $F_{1,99} = 834.3$, $P < 0.00001$). Nevertheless, lizards from colder environments hatched earlier than lizards from warmer environments at both incubation temperatures (Table 2, Fig. 4). In the western clade, individuals from IN hatched earlier than individuals from FL (MS = 475.2, $F_{1,22} = 14.76$, $P < 0.001$). In the eastern clade, individuals from NJ and VA hatched earlier than individuals from SC (OHT: MS = 660.5, $F_{2,41} = 37.18$, $r_s P_c > 0.99999$, $P < 0.00001$).

Lizards from colder environments not only hatched earlier, but also grew more efficiently at both incubation temperatures (Table 2, Fig. 5). In the western clade, embryos from IN grew

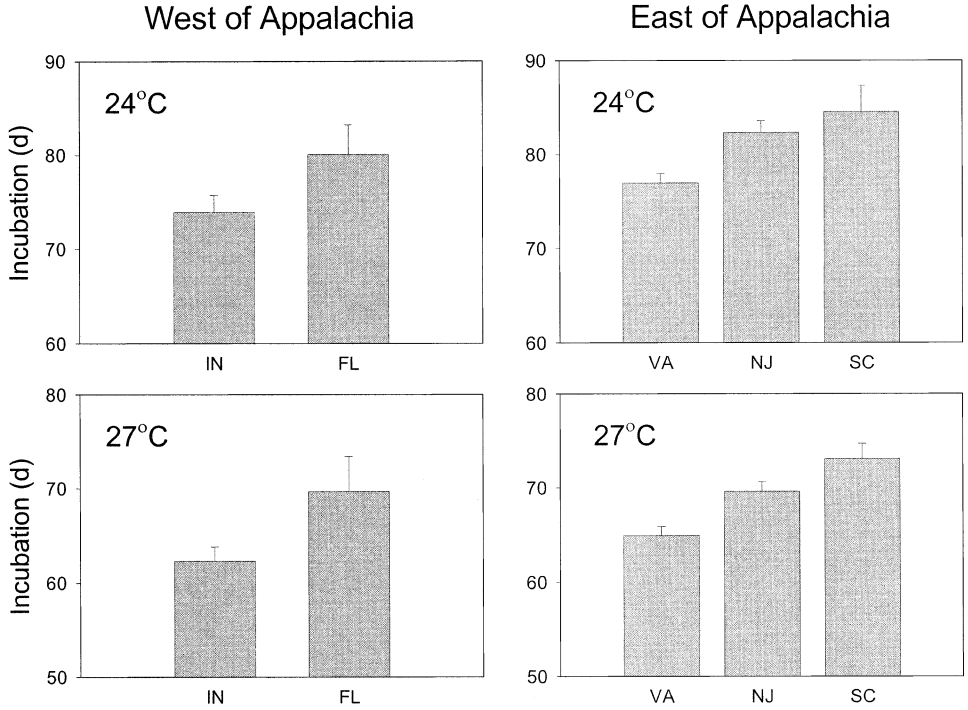

FIG. 4. In two clades of *Sceloporus undulatus*, embryos from colder environments hatched earlier than embryos from warmer environments when incubated under either thermal cycle (cool, 24°C; warm, 27°C). Error bars are 95% confidence intervals.

TABLE 2. Phenotypes of hatchlings from the five populations of *Sceloporus undulatus*. All values are listed as mean ± 95% confidence interval. Sample sizes (*n*) are the number of individuals followed by the number of clutches in parentheses.

Population	*n*	Hatching success	Mass (mg)	SVL (mm)	Incubation period (d)	Growth efficiency (%)
Indiana	47 (15)	82%	574.2 ± 20.8	26.53 ± 0.43	67.7 ± 2.0	44.6 ± 1.6
Florida	17 (8)	54%	501.7 ± 46.2	25.23 ± 0.72	74.6 ± 3.5	39.2 ± 2.6
New Jersey	58 (13)	76%	582.9 ± 15.2	26.53 ± 0.32	75.8 ± 1.9	45.3 ± 1.0
Virginia						
Unmanipulated	52 (17)	86%	604.0 ± 18.9	27.32 ± 0.33	70.9 ± 1.8	46.0 ± 1.1
Yolkectomized	28 (12)	80%	502.5 ± 25.4	25.68 ± 0.47	71.5 ± 2.4	47.1 ± 2.3
South Carolina	31 (9)	81%	527.1 ± 14.0	26.12 ± 0.36	79.0 ± 2.6	42.8 ± 1.4

more efficiently than embryos from FL (MS = 0.22, $F_{1,32}$ = 9.25, $P < 0.01$). In the eastern clade, embryos from NJ and VA grew more efficiently than embryos from SC (OHT: MS = 0.12, $F_{2,67}$ = 3.29, r_sP_c = 0.96, $P < 0.01$). Differences in growth efficiency between populations were similar between years. In the western clade, embryos from IN grew more efficiently than embryos from FL in both years (2002: MS = 0.33, $F_{1,32}$ = 13.88, $P < 0.001$; 2003: MS = 0.10, $F_{1,32}$ = 4.20, $P < 0.05$), even though the magnitude of this difference changed between years (MS = 0.11, $F_{2,32}$ = 4.64, P = 0.02). In the eastern clade, no interaction between year and population was observed (MS = 0.07, $F_{2,67}$ = 1.78, P = 0.18). Incubation temperature did not affect growth efficiency (western clade: MS = 0.01, $F_{1,32}$ = 0.26, P = 0.61; eastern clade: MS < 0.01, $F_{1,67}$ = 0.13, P = 0.72), and differences between northern and southern populations were similar at both temperatures.

Differences in hatchling size between populations were consistent with the differences in growth efficiency (Table 2). In the western clade, hatchlings from IN were heavier (MS = 0.10, $F_{1,23}$ = 9.70, $P < 0.01$) and tended to be longer (MS = 10.22, $F_{1,28}$ = 3.96, P = 0.06) than hatchlings from FL. In the eastern clade, hatchlings from VA and NJ were heavier (OHT: MS = 0.05, $F_{2,38}$ = 6.70, r_sP_c = 0.997, $P <$ 0.01) and longer (OHT: MS = 13.84, $F_{2,41}$ = 5.38, r_sP_c = 0.992, $P < 0.01$) than hatchlings from SC. Because we adjusted hatchling size for egg mass prior to our analyses, the differences in hatchling size between populations reflected different capacities for embryonic growth given similar resources.

Through yolk reduction, we were able to successfully eliminate differences in egg size between embryos from VA and SC; miniaturized eggs from VA were smaller than unmanipulated eggs from the same population but were similar in size to eggs from SC (Table 2). Still, miniaturized eggs from VA hatched earlier (MS = 735.10, $F_{1,28}$ = 55.73, $P <$ 0.000001) and grew more efficiently (MS = 0.24, $F_{1,32}$ = 8.65, $P < 0.01$) than unmanipulated eggs from SC (Table 2).

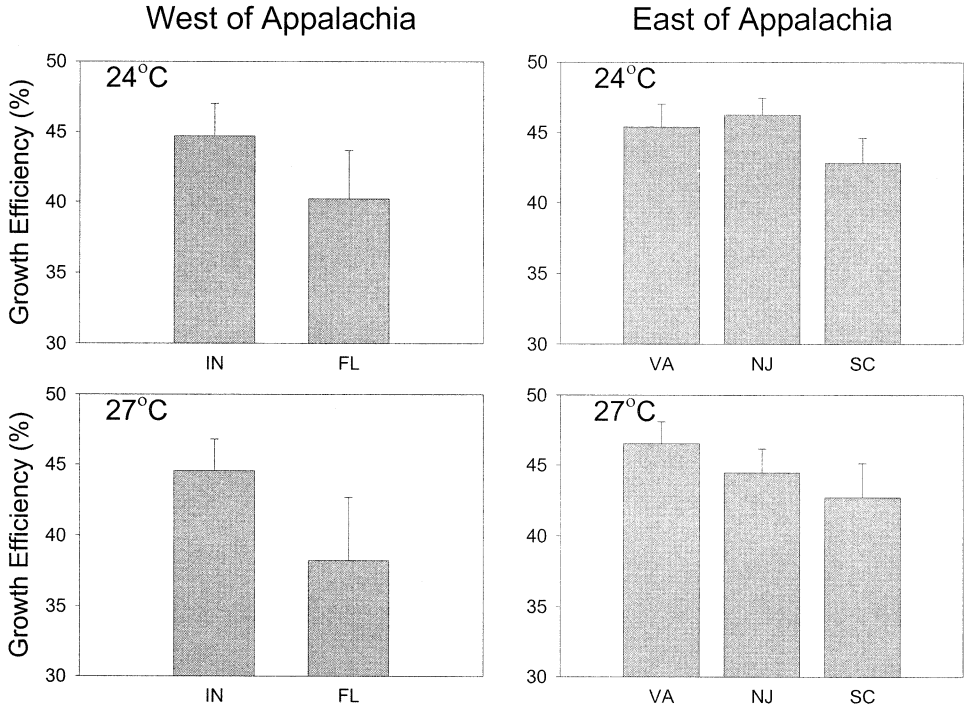

FIG. 5. In two clades of *Sceloporus undulatus*, embryos from colder environments grew more efficiently than embryos from warmer environments when incubated under either thermal cycle (cool, 24°C; warm 27°C). Error bars are 95% confidence intervals.

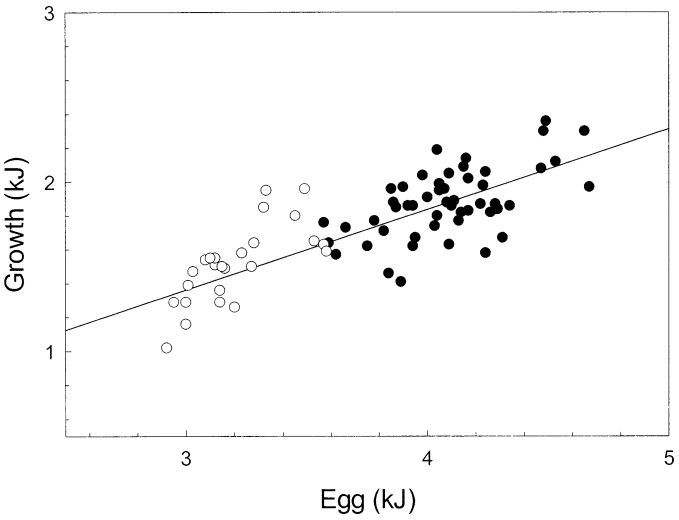

FIG. 6. The growth efficiencies of miniaturized eggs from Virginia (open circles) were similar to those of unmanipulated eggs from the same population (closed circles). The similarity of growth efficiency is indicated by the common relationship between energy availability and growth. The solid line is the regression model for unmanipulated eggs ($\beta = 0.59$, $\alpha = -0.065$, Adjusted $r^2 = 0.34$, $P < 0.00001$, $n = 52$).

Moreover, the incubation periods and growth efficiencies of miniaturized and unmanipulated eggs from VA were nearly identical (Table 2, Fig. 6). Therefore, the relatively rapid growth and development of embryos from VA was not simply a consequence of their relatively large supply of energy.

DISCUSSION

Geographic Variation in Growth and Development

When embryos of *S. undulatus* were reared in two common environments, individuals from northern populations hatched earlier and grew more efficiently than individuals from southern populations. Furthermore, these patterns were consistent between clades in both years of our study. Our observations accord with the predictions of optimality theory, as well as the observations of other investigators who have documented rapid growth and development by genotypes from cold environments. For example, tadpoles of *Rana temporiana* from northern populations developed faster than those from southern populations when reared in multiple common environments (Laugen et al. 2003a). Similarly, genotypes of *Drosophila melanogaster* from northern populations grew more rapidly during the larval period than did genotypes from southern populations (Robinson and Partridge 2001). Likewise, numerous cases of countergradient variation in the growth rates of juveniles have been documented (reviewed by Conover and Schultz 1995; Angilletta et al. 2002).

Much less attention has been focused on geographic variation in embryonic growth and development (Laugen et al. 2003b). The few available data from common garden experiments tell conflicting stories. For example, Qualls and Shine (1998) found that lizards (*Lampropholis guichenoti*) from a high elevation hatched earlier than lizards from a low elevation. In contrast, the embryonic development of frogs (*Rana temporaria*) was unrelated to the latitude or the temperature of the source environment (Laugen et al. 2003b). Interspecific comparisons have also yielded mixed evidence. Compared to its southern congener (*Coenagrion puella*), a northern species of damselfly (*Coenagrion hastulatum*) grew and developed faster at low temperatures but slower at a high temperature (Van Doorslaer and Stoks 2005). Yet species of *Sceloporus* from cold environments did not develop faster than congeners from warm environments (Andrews et al. 1999). Our study revealed countergradient variation in *Sceloporus undulatus*, even though such variation was not evident among species of the same genus.

The discrepancies among these observations could reflect the unique evolutionary histories of these species, but could also reflect differential effects of uncontrolled variables. In particular, Qualls and Shine (1998) noted a difference in incubation period between populations could be caused by a bias in the developmental stage at oviposition (developmental bias). Although available data suggest developmental bias was unlikely (see Methods), Qualls and Shine also argued the standard method of staging embryos is too coarse to detect biologically significant developmental bias. If they are correct, the only way to avoid developmental bias might be (1) to replicate observations within populations (i.e., include eggs from many females) and (2) to replicate experiments among years (i.e., sample eggs from females that experience different environmental conditions). With sufficient replication, variation in the developmental stage at oviposition becomes random error. In our experiment, we replicated the comparison of incubation periods between years and between clades. Assuming no systematic variation in the developmental stage at oviposition, the probability of observing the patterns predicted by theory in both clades during both years was 0.7%; this conclusion follows from the fact that the observed orderings of means within clades and within years was one of 144 possibilities. Furthermore, experiments conducted in 2004 and 2005 also showed that eggs from VA and NJ hatched significantly earlier than did eggs from SC (M. Blake, M. Angilletta, and P. Niewiarowski, unpubl. data). In fact, differences in incubation period between embryos from VA and embryos from SC—populations at the thermal extremes—were similar among the four years (eight, eight, six, and five days in 2002, 2003, 2004, and 2005, respectively). Altogether, these data drastically decrease the likelihood that developmental bias caused the differences in incubation period between populations of *S. undulatus*.

We can also exclude developmental bias as a plausible explanation for differences in growth efficiency between populations. Suppose embryos from cold environments were well developed at oviposition but embryos from warm environments were poorly developed. Well developed embryos would have consumed more of their available energy; consequently, growth efficiencies of these embryos would have been artificially inflated because we ignored respiration of embryos prior to oviposition. Could such developmental bias account for the differences in growth efficiency between populations? The smallest difference in growth efficiency was observed between embryos from NJ and those from SC. Based on our conservative estimate (see Methods), an extreme difference in the timing of oviposition (16 days) would only account for a 1% difference in growth efficiency; yet

the observed difference was 2.5%. Developmental bias simply cannot account for the differences in growth efficiency between populations of *S. undulatus*.

Ecological and Evolutionary Causes of Countergradient Variation

Although many cases of countergradient variation have been documented, the mechanisms that enable some genotypes to grow and/or develop faster than others are known for only a few cases. Countergradient variation can result from either genetic or environmental factors. Several studies have documented a genetic basis for countergradient variation (Conover and Present 1990; Robinson and Partridge 2001), but in some cases environmental factors are important. For example, Sears and Angilletta (2003) observed that sagebrush lizards (*Sceloporus graciosus*) at a high elevation grew faster than those at lower elevations. Nevertheless, lizards from all elevations grew at a similar rate when raised in the laboratory. They concluded acclimatization to local conditions enabled juveniles at high altitude to grow more rapidly than those at low altitude. Importantly, environmental factors include the physiological states of offspring that are influenced by the maternal environment.

One particular maternal effect—the energetic provisioning of eggs—has a major influence on the growth and development of embryos (Bernardo 1996; Mousseau and Fox 1998). Because females of *S. undulatus* from colder environments produce larger eggs (Table 2; Angilletta et al. 2005), their offspring might have grown and developed more rapidly because they had more energy. But we reduced the energy available to embryos from VA and observed no difference in incubation period or growth efficiency between these embryos and those in unmanipulated eggs. This result suggests patterns of growth and development were influenced by something other than the maternal effect of egg size. A genetic factor seems likely when one considers that genetic variation in energy assimilation and protein turnover has been linked to intraspecific variation in growth rate (Hawkins 1995; Bayne 2004). Generally, individuals that are more heterozygous at multiple loci assimilate energy more efficiently, and spend less energy on maintenance; hence, these individuals grow rapidly (Hawkins 1995; Hawkins and Day 1999). If genetic divergence of embryonic growth and development has occurred in *S. undulatus*, what are the selective pressures that maintain countergradient variation?

Natural selection of offspring size could explain the convergent evolution of countergradient variation in *S. undulatus*. Optimality models predict larger offspring in colder environments because of relationships among body size, survivorship, and fecundity (Perrin 1988; Yampolsky and Scheiner 1996; Stelzer 2002). Larger offspring can attain a minimal reproductive size earlier, possibly resulting in higher survival to maturity or greater fecundity during adulthood (Fig. 1). We suspect these optimality models apply to *S. undulatus* because both egg size and embryonic physiology seem to enhance growth in cold environments (Tables 1 and 2). Additionally, a shorter incubation period would enable offspring to grow longer before brumation, which would compensate for relatively slow growth in cold environments. To test these hypotheses, we could quantify the effects of offspring size on survival and fecundity in different thermal environments. In doing so, we could combine manipulations of egg size and reciprocal transplants of genotypes to expose the full range of phenotypes to each environment (Schluter 2000; Arnold 2003; Brooks et al. 2005).

Although theorists have considered the trade-offs associated with producing large eggs, they have generally ignored the trade-offs associated with rapid growth and development. For countergradient variation to evolve by natural selection, rapid growth and development must involve a trade-off manifested in the embryonic or juvenile stage (Gotthard 2001); otherwise, natural selection should maximize growth and development in all environments. Possibly, embryos speed growth and development by reducing cellular maintenance. If this hypothesis is correct, we might expect genotypes that grow and develop rapidly to suffer higher mortality than do genotypes that grow and develop slowly. Additionally, embryos could speed growth and development by assimilating yolk faster. This strategy should slow growth during the juvenile stage because less yolk would remain after hatching (Troyer 1987). These energetic trade-offs could be less severe than anticipated because a shorter incubation period means that embryonic tissues are maintained for a shorter duration. Importantly, embryos can speed growth and development by both mechanisms to minimize the fitness cost imposed by these trade-offs (Angilletta et al. 2003).

A broader perspective on the proximate and ultimate mechanisms that generate countergradient variation in growth and development can be gained from intraspecific studies of convergent evolution. The recent proliferation of intraspecific phylogenies and comparative methods has enabled biologists to exploit phylogenetic information in studies of microevolution (Niewiarowski et al. 2004). Such studies have revealed many examples of convergent evolution of morphology and life history within species (Reznick et al. 1996; Zamudio 1998; Wiens et al. 1999; Johnson 2001; Huey et al. 2002; Angilletta et al. 2004). Still, phylogenetic comparative methods have not been applied extensively in studies of intraspecific variation in physiology, even though such applications could help us to understand the convergence of other phenotypes (e.g., offspring size, age, and size at maturity). The recognition of convergent evolution within a species creates an opportunity to examine the generality of selective pressures, biochemical processes, and genetic mechanisms. Thus, *S. undulatus* could be a valuable complement to other organismal models (e.g., *Menidia menidia*, *Drosophila melanogaster*) when exploring the causes and consequences of countergradient variation.

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WX02169) and were treated in a manner approved by the Institutional Animal Care and Use Committee (02-4: MA/CO).

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