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Rapid assimilation of yolk enhances growth and development of lizard embryos from a cold environment

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Summary

Selection for rapid growth and development in cold environments results in a geographic pattern known as countergradient variation. The eastern fence lizard, *Sceloporus undulatus*, exhibits countergradient variation in embryonic growth and development along latitudinal clines. To identify the proximate causes of countergradient variation, we compared the energy budgets of embryos from a cold environment (Virginia) and a warm environment (South Carolina) during development at a realistic thermal cycle. The difference in mean egg size between populations was controlled by removing yolk from large eggs and performing a sham manipulation on other eggs. Respiration was measured every 4 days throughout 48 days of incubation. After this period, eggs were dissected and the energy contents of embryos and yolk were determined by calorimetry. As expected from previous experiments, embryos from Virginia reached a more advanced stage of development and deposited more energy within tissues than embryos from South Carolina. The greater absorption of yolk by embryos from Virginia was associated with a higher rate of respiration. Assimilation of yolk by rapidly growing embryos could reduce growth or survival after hatching. Such costs might explain the maintenance of countergradient variation in *S. undulatus*.

Key words: countergradient variation, growth rate, *Sceloporus*, tradeoff, yolkectomy.

Introduction

The discovery of countergradient variation in growth rate has stimulated renewed interest in the evolution of physiological capacities in both plants and animals (for reviews, see Arendt, 1997; Conover and Schultz, 1995). Most documented cases of countergradient variation (*sensu* Levins, 1968) have resulted from the evolution of a greater capacity for growth in colder environments (Berven et al., 1979; Conover et al., 1997; Conover and Present, 1990; Jonassen et al., 2000). The maintenance of a low capacity for growth in warm environments seems counterintuitive because large size can confer greater survival and fecundity (Roff, 2002; Stearns, 1992). The resolution of this paradox requires us to understand the tradeoffs between growth and other functions that affect the fitness of organisms (Billerbeck et al., 2001; Gotthard, 2001; Lankford et al., 2001). To identify potential tradeoffs, we must first determine the proximate mechanisms by which certain genotypes achieve rapid growth (Angilletta et al., 2003; Present and Conover, 1992).

Embryos constitute a simplified biological system in which to investigate the proximate sources of variation in growth rate. Most embryos reside within eggs than contain a fixed supply of resources. Moreover, embryos expend little energy on locomotion and no energy on reproduction. Consequently, embryos consume most of their available energy for growth and respiration. Countergradient variation in embryonic growth can arise in two ways: embryos from colder environments either have more energy for growth or use their energy more efficiently. In some species, mothers in colder environments provision their embryos with larger supplies of energy (Angilletta et al., 2006b; Atkinson et al., 2001). By manipulating the energy available to embryos, we can isolate the effects of maternal energy allocation and embryonic growth efficiency (Oufiero and Angilletta, 2006). Embryos could grow more efficiently by assimilating yolk more rapidly or by reducing costs of growth and maintenance. Either strategy would enhance fitness in a cold environment, where the poor potential for thermoregulation and activity limits growth after hatching (see Oufiero and Angilletta, 2006). Still, both strategies should impose an energetic tradeoff that could negatively impact the survival of the embryo or its performance after hatching. Such tradeoffs would favor submaximal growth in environments where the costs of rapid growth outweigh the benefits.

In this paper, we report the proximate causes of countergradient variation in embryonic growth of the eastern fence lizard (*Sceloporus undulatus*), with emphasis on the likely tradeoffs that constrain the evolution of growth rates along geographic clines. Recently, Oufiero and Angilletta discovered that *S. undulatus* has evolved countergradient variation at least twice along latitudinal clines (Oufiero and Angilletta, 2006). Although embryos from colder environments generally have more yolk (Angilletta et al., 2006b), countergradient variation persisted after controlling for differences in egg size between populations. From this result, Oufiero and Angilletta concluded

that the physiological capacity for growth must have diverged between populations. To determine the proximate mechanisms that underlie differences in embryonic growth efficiency, we compared the energetics of embryos from two of the populations studied previously. As in previous experiments (Sinervo, 1990; Sinervo et al., 1992), we removed yolk from eggs (yolkectomy) to manipulate the energy available to embryos and ameliorate natural differences between populations. Additionally, we controlled the duration of growth by measuring energetics during a fixed period of development. Our results clearly show that embryos from the cold environment fueled their rapid growth by assimilating more yolk, potentially leaving less yolk available for juvenile growth.

Materials and methods

All procedures described in this paper were approved by Indiana State University's Animal Care and Use Committee.

Collection and husbandry

During the spring of 2005, we collected gravid females of *Sceloporus undulatus* (Bosc and Daudin) from Edgefield County, South Carolina, USA (SC) (122–137 m) and Montgomery, Giles, and Craig Counties, Virginia, USA (VA) (884–1128 m). Mean annual air temperature of the area in SC (18.0°C) greatly exceeds that of the area in VA (11.0°C). Both populations belong to the eastern clade of *S. undulatus*, as described by Leaché and Reeder (Leaché and Reeder, 2002).

Lizards were placed individually in cloth bags and were stored in an insulated container for up to 72 h while being transported to Indiana State University. In the laboratory, lizards were placed in glass terraria (38 liters) with a substrate of fine sand. Terraria were kept in an environmental chamber set at a temperature of 25±1°C and a light cycle of 12 h:12 h L:D. An incandescent bulb (40 W) was placed at one end of each terrarium to enable lizards to thermoregulate. Lizards were fed domestic crickets (*Acheta domesticus*) to satiation every other day. Water was available at all times.

Acquisition of eggs

We acquired eggs by hormonally inducing females to oviposit. Controlling the timing of oviposition served two purposes. First, we could weigh eggs immediately after laying to avoid changes in mass caused by water flux. Second, we could coordinate dates of oviposition among females to synchronize measures of embryonic respiration. Oviposition was induced with an intracoelomic injection of ≈0.5 ml of oxytocin (20 USP; The Butler Company, Columbus, OH, USA). Although this procedure probably reduced the initial stage of development for some embryos (Parker et al., 2004), we have no reason to believe that hormonal induction would have affected mean developmental stage more in one population than the other (see Oufiero and Angilletta, 2006). After induction, each lizard was placed in a plastic container (4 liters) with a substrate of moist sand. These containers were kept in a dark incubator set at 30°C. We monitored females closely for up to nine hours or until palpation revealed that all eggs had been deposited. Freshly laid eggs were assigned unique marks for identification and were weighed to the nearest 0.01 mg.

Manipulation of egg size

Eggs received one of three treatments: (1) incubation after removal of yolk (yolkectomized eggs), (2) incubation after a sham manipulation (sham eggs) or (3) incubation without manipulation (control eggs). Females from VA generally produce larger eggs than females from SC (Oufiero and Angilletta, 2006). Therefore, eggs from VA received yolkectomy, sham and control treatments (12, 13 and 11 eggs, respectively), but eggs from SC received only sham and control treatments (17 and 19 eggs, respectively). To avoid pseudoreplication, only one egg from each clutch was randomly assigned to each treatment; uneven sample sizes reflect mortality of eggs in some treatments. Additionally, one egg from each clutch was frozen at –60°C on the day of oviposition. These eggs were used to estimate the energy available to embryos (see below). To yolkectomize eggs, we used a syringe to aspirate 30–100 mg of yolk (60±20 mg, mean ± s.d.), depending on the initial mass of the egg. Sham eggs were pierced with a needle but no yolk was removed. Control eggs were handled briefly but were incubated without further manipulation.

Incubation of eggs

Eggs were incubated in plastic containers (10×10×6 cm) containing a substrate of fine sand (100% silica). The water content of the sand was maintained at 1% of total mass, yielding a water potential of –10 kPa (Oufiero and Angilletta, 2006). To avoid conflating effects of incubation and source environments, no more than one egg from each population was incubated in each container. These containers were kept in two programmable incubators (Model KB 115; Brinkman Instruments, Westbury, NY, USA), which maintained a daily cycle of temperatures ranging from 20 to 34°C [see fig. 3 of (Oufiero and Angilletta, 2006)]. This thermal cycle resembles those of natural nests in New Jersey and Virginia (Angilletta et al., 2005; Warner and Andrews, 2002). Although we do not know the temperatures of nests in SC, differences in growth and development between populations did not depend on incubation temperature in a previous experiment (Oufiero and Angilletta, 2006). We shuffled the containers within and between incubators every 2 days to minimize effects of thermal gradients; nevertheless, gradients in temperature within each incubator were trivial (≤0.5°C), as verified by temperature loggers that we placed inside containers (Thermochron iButton; Maxim Integrated Products, Sunnyvale, CA, USA). Every 4 days, we replaced water that had evaporated from each container to minimize changes in water potential during development.

After 48 days of incubation, eggs were stored at –60°C until we could determine developmental stages and energy contents. We chose to end development after 48 days for two reasons. First, metabolic rate increases approximately linearly during this period, which simplified our models of embryonic respiration (see below). Second, embryos from both populations require more than 48 days to complete development, which ensured that no lizards would hatch before the end of our experiment. Mean incubation periods of sister embryos from VA and SC were 66.9±3.1 days and 76.0±2.2 days, respectively (P. H. Niewiarowski, M.J.A. and M.A.S., manuscript in review),

which suggests that embryos from VA and SC completed approximately 72% and 63% of their incubation period during the experiment.

Embryonic respiration

For a subset of embryos, we estimated the energy expended on maintenance and development from measures of respiration. At set intervals throughout incubation, we recorded the oxygen consumption of embryos by closed-system respirometry (Model TR-3; Sable Systems, Las Vegas, NV, USA). These recordings began one day after oviposition and were repeated every 4 days until eggs were sacrificed.

Each recording lasted 24 h, such that respiration could be summed over an entire thermal cycle. Prior to a recording, eggs were removed from their containers, cleaned of adhering sand and weighed to the nearest 0.01 mg. Each egg was placed on a piece of sterile cotton in a plastic dish. To prevent dehydration during respirometry, 1 ml of distilled water was added to the cotton. The dish was then positioned inside a clean, glass chamber (250 ml). Sealed chambers were placed in the same programmable incubators that housed the incubation containers. To ensure that embryonic temperatures equaled the air temperature of the incubator, chambers were placed in the incubators at least 1 h prior to measures of respiration. During this time, we calibrated the oxygen analyzer (Model FC-1; Sable Systems) with a gas of known concentration. Recordings always commenced at 16.00 h. Initially, all chambers were flushed sequentially with air purged of water and carbon dioxide by a gas generator (Model 75-45; Parker Hannifin Corp., Haverhill, MA, USA). Each chamber was flushed again at 12 and 24 h after the initial flush. At each flush, all air from the chamber was passed through the oxygen analyzer at a known rate. A computer controlled the sequential flushing of chambers to ensure that each chamber was sealed for a precise duration between recordings. Given our measures of oxygen concentration and flow rate, we could calculate the total volume of oxygen consumed while the chamber was sealed. Daily energy expenditure was calculated as the sum of oxygen consumption during the sequential 12-h periods. Although each recording ended at 04.00 h, eggs were not returned to their incubation containers until 08.00–10.00 h. Thus, eggs spent about 40 h within chambers during each recording. Nevertheless, these eggs developed similarly to eggs incubated without measures of respiration.

Total respiration was estimated by integrating rates of energy expenditure over incubation. Each recording was analyzed by a computer program (CONVOL; Sable Systems) to generate a daily rate of oxygen consumption (ml day^{-1}). These rates were converted to energy expenditure (J day^{-1}) by assuming that embryos catabolized protein and lipid in equal quantities [19.05 J ml^{-1} of O_2 (Nagy, 1983)] – an assumption supported by the energy densities of eggs (Oufiero et al., 2007) and the respiratory quotients of embryos (Thompson and Russell, 1998; Thompson and Russell, 1999; Thompson and Stewart, 1997). Because we made repeated measures of respiration, we fit a linear model to each embryo's change in daily respiration. All models described respiration extremely well (median r^2=0.95; range=0.70–0.99). By integrating the fitted models, we obtained each embryo's total respiration during incubation (J).

In our analyses, we included only eggs that appeared to contain healthy embryos throughout incubation. For the population from VA, we included 7, 8 and 5 eggs from the yolkectomy, sham and control treatments, respectively. For the population from SC, we included 9 and 10 eggs from the sham and control treatments, respectively.

Developmental stages

After 48 days of incubation, we determined the developmental stages of embryos by microscopy. Eggs were warmed from –60 to –2°C in pre-weighed aluminum pans. Each egg was cut superficially on one side, and the shell was separated from the embryo and membranes. Once the embryo had thawed to room temperature, the yolk sac and chorioallantoic membrane were separated from the embryo. Because eggs frozen at oviposition lacked discernable embryos, these eggs were separated into shell and internal contents only. Shells, yolk sacs and embryos were weighed individually to the nearest 0.01 mg. Embryos were placed on ice and were examined with a dissecting microscope (Model DP12; Olympus, Center Valley, PA, USA). Developmental stages were scored using the system of DuFaure and Hubert (DuFaure and Hubert, 1961). Because some embryos were between discrete stages, we scored development to the nearest half stage. Scoring by half stages was based on the following criteria: (1) the degree to which digits and scales had developed on the hands, (2) the degree to which pigment and scales had developed on the head and (3) the degree to which eyelids had formed. Embryos at half-stages displayed either incomplete development of stage-defining characteristics or a combination of characteristics from two sequential stages. After dissection, the components of each egg were dried to a constant mass at 50°C.

Energetics of embryos

To estimate the assimilation and expenditure of energy, we determined the caloric contents of yolk sacs and embryonic tissues *via* bomb calorimetry. Dry samples were homogenized and pressed into one or two pellets weighing at least 20 mg. Prior to calorimetry, these pellets were stored in nitrogen-filled vials at –60°C. Some samples of embryonic tissue were too small to combust directly (<20 mg); these samples were mixed with benzoic acid to yield samples that were large enough to combust. After combusting the mixed samples, we used their energy density and the energy density of benzoic acid to calculate the energy density of the embryo. Shells were not formed into pellets because they contained mostly minerals that would not combust. Samples were combusted in a semimicro bomb calorimeter (Model 1420; Parr Instruments Company, Moline, IL, USA). The calorimeter was calibrated with benzoic acid several times per day. After combustion, the energy density of each sample was multiplied by its dry mass to yield its energy content. Hereafter, we refer to the energy contents of embryos and yolk as embryonic growth and residual yolk, respectively.

The energy available to each embryo was estimated from calorimetry of the eggs frozen at oviposition. Because we froze one egg from each clutch, we could use its yolk to estimate the energy available to its siblings. This method avoids the accumulation of error inherent in summing components of an

energy budget (Thompson et al., 2001) and provides accurate estimates of energy availability in *S. undulatus* (dry masses generally differ by less than 5% between sister eggs; C. E. Oufiero and M.J.A., unpublished data). To estimate the energy available to a yolkectomized egg, we assumed yolk comprised equal quantities of lipid and protein (Thompson and Speake, 2002) and calculated the energy removed by yolkectomy (see Oufiero and Angilletta, 2006).

Statistical analyses

We used analysis of covariance (ANCOVA) to examine the effects of population on developmental stage, embryonic growth and residual yolk. In each model, the effect of treatment (yolkectomy, sham or control) was nested within the effect of population (VA or SC). The initial energy in each egg was used as a covariate. A similar analysis was used to compare respiration between populations and among treatments. For our comparison of total respiration (J), we used embryonic growth (J) as a covariate; therefore, a significant difference between populations would indicate a difference in the cost of growth, defined as the slope of the relationship between growth and respiration (Wieser, 1994). Additionally, we compared rates of respiration near the end of incubation (day 45), using embryonic dry mass as a covariate. Prior to each analysis, we assessed whether slopes of the relationship between the covariate and the dependent variable differed between populations; when slopes differed, we based our conclusions on a model that included the interaction between the effects of the covariate and the population (see Engqvist, 2005). All analyses were performed with Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). Descriptive statistics are reported as means ± 95% confidence intervals.

Results

To compare the growth and development of embryos from VA and SC over a similar range of egg sizes, we yolkectomized some eggs from VA and sham-manipulated other eggs from VA and SC. Yolkectomy reduced the mean egg mass from 0.43 g (±0.01) to 0.37 g, which was identical to the mean mass of control eggs from SC (0.37±0.01 g). We were primarily interested in the effect of population, but we also wished to know whether our manipulation of egg size introduced artifacts in our results. Because we used initial energy as a covariate, a significant effect of treatment would mean that embryos in small eggs created by yolkectomy differed from embryos in naturally small eggs. Fortunately, neither developmental stage ($F_{3,66}$=0.33, P=0.80) nor embryonic growth ($F_{3,66}$=0.88, P=0.46) differed among treatments. Furthermore, manipulations of egg size did not affect the total respiration during incubation ($F_{3,33}$=0.18, P=0.91) or the respiration on day 45 of incubation (separate slopes ANCOVA; $F_{3,31}$=0.42, P=0.74). Thus, neither actual nor sham manipulation of egg size created artifacts that should influence our interpretation of the data.

As expected from previous experiments (Oufiero and Angilletta, 2006), embryos from VA developed and grew more rapidly than embryos from SC, even when both had similar quantities of energy at oviposition (see Fig. 1 and Fig. 2). Embryos from VA were about half a stage more developed after 48 days of incubation than were embryos from SC ($F_{1,66}$=5.74, P=0.02). In other words, embryos from VA had better-defined scales on their hands, more pigmentation on their fingers and greater development of their eyelids. Furthermore, embryos from VA contained 60% more energy in their tissues than embryos from SC ($F_{1,66}$=28.99, P<0.00001). The relatively rapid growth of embryos from VA resulted from a greater assimilation of yolk rather than a reduction in maintenance. Embryos from VA had less residual yolk after 48 days of incubation than embryos from SC (Fig. 3), even after adjusting residual yolk for initial energy ($F_{1,66}$=15.45, P<0.001). The difference in mean residual yolk between populations was nearly energetically equivalent to the difference in growth (see Fig. 2 and Fig. 3). Still, the rapid assimilation of energy by embryos from VA appears to have imposed an energetic cost (Fig. 4). Based on our ANCOVA, the 'cost of growth' (*sensu* Wieser, 1994) was approximately half a Joule of respiration per Joule of tissue (β=0.57±0.27; $F_{1,33}$=19.12, P<0.001). But even

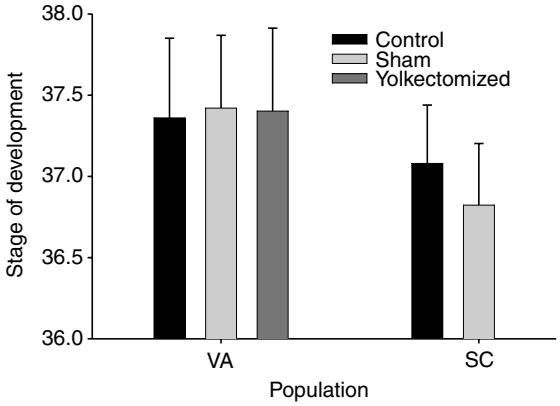

Fig. 1. Embryos from VA developed more rapidly than embryos from SC. After 48 days of incubation, embryos from VA (N=36) and SC (N=36) reached stages 37.4±0.3 and 37.0±0.3, respectively. Yolk manipulation did not affect the development of embryos. Data were adjusted for initial energy by ANCOVA. Error bars represent 95% confidence intervals of means for each treatment.

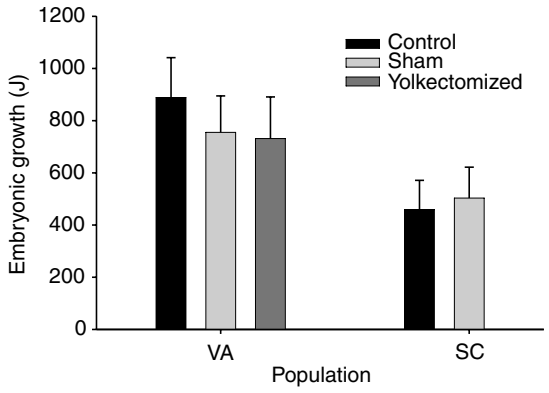

Fig. 2. Embryos from VA grew more rapidly than embryos from SC. Mean investments in growth were 792±76 J and 481±58 J for embryos from VA (N=36) and SC (N=36), respectively. Yolk manipulation did not affect the growth of embryos. Data were adjusted for initial energy by ANCOVA. Error bars represent 95% confidence intervals of means for each treatment.

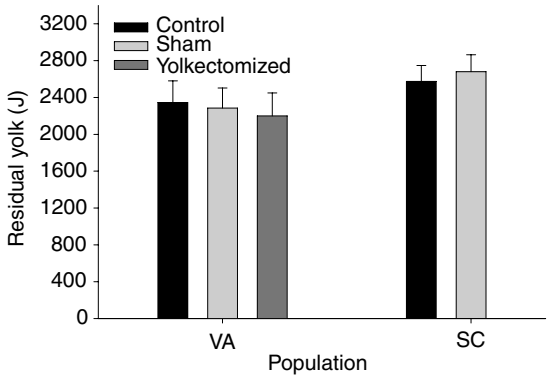

Fig. 3. Embryos from VA assimilated more yolk than embryos from SC. Mean quantities of residual yolk were 2277±126 J and 2628±126 J for embryos from VA (*N*=36) and SC (*N*=36), respectively. Yolk manipulation did not affect the assimilation of yolk by embryos. Data were adjusted for initial energy by ANCOVA. Error bars represent 95% confidence intervals of means for each treatment.

after we adjusted for variation in embryonic growth, the total respiration of embryos from VA exceeded that of embryos from SC ($F_{1,33}$=6.79, P=0.01). Moreover, embryos from VA respired more than embryos from SC on day 45 (separate slopes ANCOVA; $F_{1,31}$=5.34, P=0.03). Because both comparisons were made after adjusting respiration for embryonic growth, these differences indicate a relatively high cost of growth for the rapidly growing embryos from VA.

Discussion

Our results confirm the existence of countergradient variation in the embryonic growth and development of *S. undulatus*. Previously, countergradient variation in developmental rate was inferred from comparisons of incubation periods (Oufiero and Angilletta, 2006). However, incubation periods may not reflect developmental rates because an embryo could remain in the egg after reaching its final developmental stage. By directly measuring development during a fixed period, we found that embryos from a cold environment (VA) had reached a later stage than embryos from a warm environment (SC). Moreover, embryos from VA had deposited more energy within their tissues than embryos from SC. This countergradient variation in growth and development resembles that documented in other species of animals, including insects (Neat et al., 1995), crustaceans (Lonsdale and Levinton, 1985), fish (Conover, 1990; Imsland et al., 2000b; Purchase and Brown, 2000), amphibians (Berven, 1982) and reptiles (Qualls and Shine, 1998). For most of these species, biologists have yet to identify the proximate and ultimate mechanisms that gave rise to these patterns.

Two proximate mechanisms can lead to countergradient variation along thermal clines: genotypes from cold environments either assimilate more energy or grow more efficiently than genotypes from warm environments. In certain species, we know that greater assimilation by rapidly growing genotypes generates countergradient variation (Conover and Present, 1990; Jonassen et al., 2000; Nicieza et al., 1994). In other species, this phenomenon appears to result from

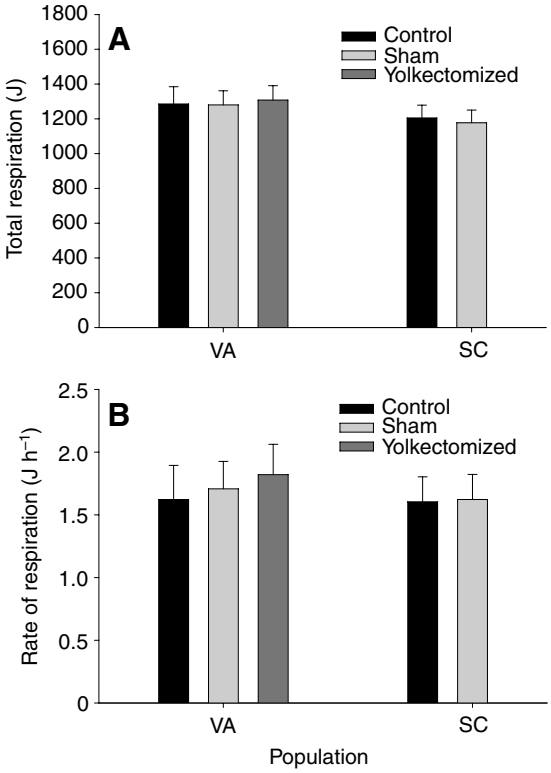

Fig. 4. Both the total respiration (A) and respiration during day 45 (B) were greater for embryos from VA than they were for embryos from SC. Total respiration was 1291±52 J and 1191±53 J for embryos from VA (*N*=20) and SC (*N*=19), respectively. Respiration during day 45 was 1.71±0.13 J h^{-1} and 1.62±0.13 J h^{-1} for embryos from VA (*N*=19) and SC (*N*=19), respectively. Yolk manipulation did not affect the respiration of embryos. Data were adjusted for embryonic growth or embryonic mass by ANCOVA (see text for details). Error bars represent 95% confidence intervals of means for each treatment.

differences in the allocation of energy between growth and maintenance (Imsland et al., 2000a; Imsland et al., 2000b; Malloy and Targett, 1994; Neat et al., 1995). In *S. undulatus*, embryos from VA grew faster than embryos from SC by assimilating energy more rapidly. Rapid assimilation of yolk by embryos from VA could explain their correspondingly high rates of respiration. During the 48 days of incubation, embryos from VA expended 8% more energy on respiration than embryos from SC (see Fig. 4A). Presumably, a relatively high rate of respiration reflects a greater need to generate ATP for maintenance, development and growth. For example, rapid growth and development should require extensive extra-embryonic membranes for transferring nutrients and wastes. The maintenance and function of membranes directly limits the capacity for anabolism and constitutes a major cost of living (Else and Hulbert, 2003). Other likely sources of variation in respiration between populations include protein turnover and tissue remodeling associated with development.

Although metabolic costs were relatively high for rapidly growing embryos, a reduction in the incubation period resulting from rapid development helps to compensate for this expense. In other words, an individual that hatches earlier will need less energy to maintain itself as an embryo (Angilletta et

al., 2006a). We can use our measures of respiration to conservatively estimate the energy saved by rapid development. Towards the end of the 48 days of incubation, embryos from VA expended about 40 J day^{-1}. Because lizards from VA complete incubation as many as 8 days earlier than lizards from SC do (Oufiero and Angilletta, 2006), the savings could amount to 320 J. This conservative estimate does not account for the fact that rates of respiration during development would ultimately exceed 40 J day^{-1} (Angilletta et al., 2000). To put this energetic saving in perspective, the mean respiration of an embryo from VA exceeded that of an embryo from SC by only 100 J during the first 48 days of incubation. Therefore, we believe the energetic savings resulting from rapid development more than compensate for the higher rate of energy expenditure.

High rates of energy assimilation by embryos should lead to tradeoffs that affect fitness. In other life stages, greater assimilation carries a cost of predation risk (Gotthard, 2000; Lankford et al., 2001). In embryos, costs of assimilation could arise in different ways. For example, residual yolk can enhance growth or survival after hatching (Ji and Sun, 2000; Ji et al., 1997; Pandav et al., 2006; Troyer, 1987). In a companion study (P. H. Niewiarowski, M.J.A. and M.A.S., manuscript in review), we measured the growth of hatchlings from VA and SC over a period of 60 days. We found that lizards from VA grew slower after hatching than lizards from SC. To our surprise, this difference between populations was inconsistent with the variation among individuals; within both populations, lizards that grew rapidly as embryos also grew rapidly as hatchlings. At present, we do not know the cause of these complex patterns. Nevertheless, we suspect the tradeoffs imposed by rapid embryonic growth occur primarily in natural environments. Hatchlings with low energy reserves would need to forage more intensely during the first few days of life, when their inexperience with predators and ignorance of their surroundings make them particularly vulnerable. This cost of embryonic growth cannot be observed in an artificial environment with unlimited food and no predators.

If rapid embryonic growth does impose a cost, we should detect this cost through field experiments. First, we could release hatchlings from VA and SC into natural environments and compare their growth and survival (Sinervo, 1990; Warner and Andrews, 2003). Second, we could directly manipulate the residual yolk of hatchlings to assess whether energy assimilation during the embryonic stage trades off with physiological performance during the juvenile stage. By directly manipulating residual yolk, rather than egg size, we could avoid confounding the effects of residual yolk and hatchling size. For example, Troyer cleverly removed iguanas from their shells and tied off their yolk sac to prevent further absorption; these manipulated animals grew less than sham-manipulated and unmanipulated animals (Troyer, 1987). Similarly, Radder and colleagues (Radder et al., 2007) surgically removed residual yolk from hatchling lizards. In contrast to Troyer (Troyer, 1987), these investigators observed no differences in growth among manipulated, sham-manipulated and unmanipulated lizards. Unfortunately, these procedures only decrease residual yolk, whereas manipulations of egg size could either increase or decrease residual yolk. Still, we could reduce the residual yolk of lizards from SC to determine whether manipulated individuals suffer poor performance. Such experiments could reveal tradeoffs between embryonic growth and juvenile performance that maintain countergradient variation.

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