

Countergradient variation in embryonic growth and development: do embryonic and juvenile performances trade off?

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Summary

1. Countergradient variation in growth rate requires that rapid growth rate trades off with other performances, such that submaximal growth evolves in certain environments. Negative effects of rapid growth on other traits within a single ontogenetic stage are conspicuous candidates for such trade-offs, but trade-offs spanning ontogenetic stages have received much less attention.
2. We tested whether rapid growth and development of embryonic lizards was associated with poor juvenile performance, as estimated by growth rate and sprint speed after hatching. To do so, we raised lizards from three populations that differ in their environmental temperature and measured their performances during embryonic and hatchling stages.
3. Under the same environmental conditions, embryos from two cold environments grew and developed more rapidly than did the embryos from a warm environment. Among populations, rapid growth and development was associated with slow growth after hatching. But surprisingly, the opposite pattern was observed within populations.
4. Our results highlight the need to consider trade-offs mediated by ecological factors (e.g. competition and predation), which calls for similar experiments in natural environments.

Key-words: growth rate, life history, Phenotypic integration, *Sceloporus undulatus*, sprint speed

Introduction

When genetic influences on a phenotype oppose environmental influences, a geographical pattern referred to as countergradient variation results (Conover & Present 1990; Arnott, Chiba & Conover 2006). Countergradient variation can lead to similar phenotypes along an environmental gradient because genetic variation masks environmental variation (Conover & Schultz 1995; Gotthard 2001). Countergradient variation calls attention to what should be obvious, yet is frequently unexpected by researchers: phenotypes generally reflect the optimization of multiple traits not the maximization of a single trait. Indeed, life-history theory was formulated according to a principle of optimization, where an individual trait may be constrained from reaching its theoretical maximum due to trade-offs between traits (Conover & Present 1990; Stearns 1992; Roff 2002). In other words, natural selection maximizes fitness as a function of the entire phenotype. Countergradient variation in growth rate provides an illuminating example. If we assume that growth rate has an

overwhelming effect on fitness, we might expect that maximizing growth rate would also maximize fitness. However, as Arendt (1997) emphasized, subsequent studies reinforced (Arnott *et al.* 2006), and theory has begun to address (Mangel & Stamps 2001), most organisms grow more slowly than their physiological maxima.

Potential constraints on growth rate can take many forms but recent work suggests that two mechanisms are particularly important. First, when rapid growth increases the risk of predation (Biro *et al.* 2006), natural selection can favour submaximal growth. Second, studies of compensatory growth have revealed that organisms sometimes divert energy to growth at the expense of other performances related to survival and fecundity. We draw these two examples from a large set of potential trade-offs that constrain growth rates (Mangel & Stamps 2001). Indeed, many trade-offs can cause the costs of rapid growth to outweigh the benefits (Metcalf & Monaghan 2001). Such trade-offs represent the core of life-history theory, which provides a general framework for understanding how growth rate evolves in the context of the entire life cycle (Angilletta, Steury & Sears 2004; Angilletta, Oufiero & Leache 2006b).

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In this article, we extend a previous study that revealed countergradient variation in embryonic growth and development of the eastern fence lizard, *Sceloporus undulatus*. We ask whether countergradient variation evolved because of trade-offs between embryonic and juvenile traits. Specifically, lizards from colder environments lay larger eggs that sustain faster growth and development of embryos (Warner & Andrews 2003; Niewiarowski, Angilletta & Leache 2004; Oufiero & Angilletta 2006b). Importantly, variation in growth and development persisted even after differences in egg size were eliminated by yolkectomy (Oufiero & Angilletta 2006). All else being equal, embryos from southern populations should grow and develop as rapidly as those from northern populations unless the resulting costs differ between environments (Conover & Schultz 1995; Gotthard 2001). Two, complementary explanations could account for the evolutionary divergence of growth and development between populations. First, trade-offs associated with rapid growth and development could differ among populations. Second, different environments could favour different growth rates despite identical trade-offs between embryonic physiology and other traits. In other words, the phenotypic trade-off could be driven by genetic, environmental, or interactive effects. Whatever its source, if rapid embryonic growth and development have different consequences for juvenile traits expressed in different environments, then this strategy would not be favoured in all environments. To distinguish between these alternative explanations, we must determine whether the covariation among embryonic physiology and other traits differs between lizards from cold and warm environments. If the covariation does not differ, we can reject the first explanation, at least in terms of the specific traits examined. We can then investigate whether countergradient variation evolved, because the optimal phenotype varies among environments.

If the allocation of energy to rapid embryonic growth and development limits the energy available for juveniles, a trade-off between embryonic and juvenile performances would result (Gotthard 2004; Munch & Conover 2004; Fischer *et al.* 2005; Royle, Metcalfe & Lindstrom 2006). Although many juvenile traits could trade off with embryonic growth and development, we restricted our attention to the growth rate and locomotor performance of hatchlings. These traits integrate many physiological and morphological systems and should indicate overall quality of the juvenile phenotype (Bennett 1978; Sinervo 1990). Moreover, both measures are frequently considered correlates of fitness (see Angilletta, Hill & Robson 2002). Specifically, we asked whether rapid growth and development by embryos from three populations (New Jersey, Virginia and South Carolina) comes at the expense of juvenile performance. We incubated eggs and reared hatchlings in a common garden, while employing the techniques of allometric engineering to control for differences in egg size among populations (Oufiero & Angilletta 2006a). Our experiment enabled us to test (i) whether embryonic growth and development negatively covary with juvenile growth and locomotion, and (ii) whether similar patterns of phenotypic covariation exist within and among populations.

Materials and methods

All procedures were in accordance with the regulations of the Animal Care and Use Committees of The University of Akron and Indiana State University.

COLLECTION AND HUSBANDRY

During the summer of 2005, we collected gravid females of *S. undulatus* from the following sites: (i) Edgefield County, South Carolina (SC; 34°N and 122–137 m); (ii) Montgomery, Giles, and Craig Counties, Virginia (VA, 37°N and 884–1128 m); and Burlington County, New Jersey (NJ, 40°N and 30–50 m). All three populations belong to the eastern clade of *S. undulatus*, described by Leaché & Reeder (2002). Lizards were placed individually in cloth bags and were stored in a cooler (15–26 °C) for up to 72 h while being transported to Indiana State University. Once in the laboratory, lizards were placed in glass terraria (38 L) with a substrate of sand. Terraria were kept in a room with a temperature of 25 ± 1 °C and a light cycle of 12L : 12D. Each terrarium was equipped with a 40-W incandescent light at one end to enable lizards to behaviourally thermoregulate. Lizards were fed domestic crickets (*Acheta domesticus*) to satiation every other day and were provided water *ad libitum*.

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 energetics during development (reported by Storm & Angilletta 2007). Oviposition was induced with an intracoelomic injection of approximately 0.5 mL of oxytocin (20 USP; The Butler Company, Columbus, OH). Although this procedure likely reduced the initial stage of development for some embryos (Parker, Andrews & Mathies 2004), we have no reason to believe hormonal induction would have affected mean developmental stage more in one population than another (see Oufiero & Angilletta 2006). After induction, each lizard was placed in a plastic container (4 L) with a substrate of moist sand. These containers were kept at 30 °C in a dark incubator. We checked females every 10–15 min for up to 9 h 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 were assigned to one of three treatments: (i) removal of yolk (yolkectomy), and (ii) shammed removal of yolk (shammed yolkectomy), and (iii) handling without further manipulation (control eggs). Eggs were randomly assigned to treatments with the constraint that each treatment received an equal number of eggs from each clutch. Because eggs from SC already contained relatively little yolk (Oufiero & Angilletta 2006), these eggs were only assigned to shammed yolkectomy and control treatments. To yolkectomize eggs, we used a syringe to aspirate between 0.02 and 0.1 g of yolk, depending on the initial egg mass. Sham-manipulated eggs were pierced with a syringe needle but no yolk was removed. Control eggs were incubated after a few minutes of handling to simulate yolkectomy.

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 & Angilletta 2006). To avoid confounding effects of incubation conditions and source population, each box contained only one egg from each population. Boxes were kept in incubators (Model KB 115, Brinkman Instruments, Westbury, NY), which maintained a daily cycle of temperatures ranging from 20° to 34 °C (see fig. 2 of Oufiero & Angilletta 2006). This cycle mimicked temperatures of natural nests in New Jersey (Angilletta, Oufiero & Sears 2005). We shuffled the positions of boxes within and between incubators every 2 days to avoid artefacts caused by thermal gradients. Every 4 days, we replaced water that evaporated from each box to maintain a relatively constant water potential throughout incubation. Toward the end of incubation, boxes were checked daily for hatchlings.

MEASUREMENTS OF JUVENILE PERFORMANCE

After hatching, lizards were housed individually in plastic shoeboxes (28 × 15 × 10 cm). A thermal gradient inside each shoebox enabled lizards to behaviourally thermoregulate; operative temperatures ranged from 20° to 37 °C at all times (Niewiarowski 1995). Shoeboxes were illuminated by full-spectrum lights, synchronized with the local photoperiod. Lizards were offered crickets (dusted with Reptocal™) daily and water was available continuously.

We measured growth rates and locomotor performances of juveniles. Growth rates were estimated as changes in body mass and snout-vent length (SVL) between 0 and 60 days after hatching. Locomotor performance was estimated as maximal sprint speed on day 60. Sprint speeds were measured using a 2-m racetrack, fitted with eight pairs of computer-controlled sensors. Sensors were used to calculate velocities at 0.25-m intervals. Each hatchling was raced three times, with a 1.5-h rest between races. During periods of rest, lizards were kept in an incubator set at 32.5 °C (mean field body temperature; (Angilletta *et al.* 2002). Lizards were raced immediately after being removed from the incubator.

STATISTICAL ANALYSIS

Our analyses focused on two aspects of the data. First, we used a multivariate analysis of variance (MANOVA) to determine if the four dependent variables (incubation period, size at hatching, growth rate after hatching, and maximal sprint speed) differed significantly among populations and treatments. Because each population did not receive all treatments, the effect of treatment was nested within populations. Initially, we carried out analyses of size based on mass or SVL. Because these analyses yielded qualitatively identical results, we report and discuss analyses based on length only. Based on the results of the MANOVA, we used univariate analyses to further explore variation in hatchling traits. When sprint speed was included as a dependent variable in any analysis, we used the residuals of sprint speed regressed on SVL. In accordance with the assumptions of ANCOVA, the slopes of these relationships were homogeneous among populations. Residuals were also inspected for departure from normality and the original variables were transformed if necessary.

To compare the covariance structure of the dependent variables, we used common principal components analysis (CPCA). In our

CPCA, we followed the flury step-up approach to model building (Phillips & Arnold 1999), which enabled us to test specific hypotheses of structural similarity in a hierarchical fashion, starting with the lowest level of similarity possible (one common component vs. two unrelated matrices; CPC(1)-unrelated) and proceeding to the highest level possible (matrix equality; Equal-Proportional). We judged the level of similarity using an information theoretic approach (i.e. ranking based on AIC). The CPCA was conducted using the covariance matrix for the four traits in our MANOVA. The CPCA identified whether the three populations shared the covariance structure of the dependent variables. Although MANOVA and CPCA are related, they reveal different aspects of phenotypic covariance within and among populations. The MANOVA helped us to identify how trait means differed among populations, while the CPCA helped us to identify potential constraints on the evolution of traits within populations (i.e. phenotypic trade-offs). Because multiple lizards from the same clutch were included in each treatment, we avoided pseudoreplication by averaging data within clutches prior to our analyses. Finally, survivorship was analyzed with a contingency table analysis of number of survivors in each population, stratified by treatment.

Results

Survivorship from egg to hatching (NJ = 75%; SC = 56%; VA = 73%) differed significantly among populations ($\chi^2 = 8.5$, $P = 0.015$) because of the lower survivorship of eggs from SC. Nonetheless, no significant differences in survivorship were detected among treatments within populations ($\chi^2 = 0.05$, $P = 0.82$). In addition, survivorship from hatching to 60 days of age (NJ = 59%; SC = 76%; VA = 57%) did not differ significantly among populations ($\chi^2 = 3.93$, $P = 0.14$) or treatments within populations ($\chi^2 = 0.07$, $P = 0.80$). For the results reported below, we included only those animals that survived to the age of 60 days after hatching; including individuals that did not survive the duration of the experiment would not have changed the mean values for embryonic traits, but would have precluded analyses of juvenile traits.

Yolkectomy successfully reduced the size of naturally large eggs from NJ and VA, such that mean egg masses were similar among populations ($F_{2,39} = 1.7$, $P = 0.19$; Table 1). The MANOVA yielded a significant model, in which population and treatment contributed to variation in incubation period, hatchling SVL, maximum sprint speed and growth rate (Wilks' $\lambda = 0.25$, overall model approximate $F_{28,113} = 1.88$, $P = 0.0109$). Population had a significant effect ($F_{8,62} = 4.90$, $P = 0.0001$), but treatment did not ($F_{20,104} = 0.77$, $P = 0.77$). Because we primarily wanted to know whether our manipulation of egg size introduced artefacts, we removed treatment from the model and refit the MANOVA. Excluding treatment from the final model simplified interpretation of the differences in traits among populations (see below; Fig. 2).

The MANOVA revealed significant heterogeneity of means among populations (MANOVA, Wilks' $\lambda = 0.4$, approximate $F_{8,72} = 5.2$, $P = 0.0001$), driven mainly by differences in incubation period and juvenile growth among populations (Table 1). These differences are reflected by the clustering of phenotypes in multivariate space: the phenotypes of NJ and

Table 1. Characteristics of eggs, embryos and juveniles from three populations of *Sceloporus undulatus*. Values are means \pm 1 SE. Eggs from New Jersey and Virginia included those reduced in size by yolkectomy (see text for methodological details). Sprint speed and growth in length were assayed at an age of 60 days. Unadjusted sprint speeds are given here, but size-adjusted residuals were used for statistical analyses. Statistical differences reported in the text are based on the ANOVA for each trait after a significant difference among populations was detected with a MANOVA

Population	<i>n</i> †	Egg mass (g)	Hatchling SVL (mm)	Incubation period (day)	Sprint speed (cm/s)	Growth (mm)
NJ	13	0.42 \pm 0.008	25.5 \pm 0.20	67.3 \pm 1.00	75.9 \pm 7.4	7.5 \pm 0.60
SC	19	0.39 \pm 0.010	24.7 \pm 0.34	72.1 \pm 0.67	75.9 \pm 5.8	9.3 \pm 0.56
VA	10	0.40 \pm 0.020	24.8 \pm 0.28	66.7 \pm 0.84	69.7 \pm 5.7	6.9 \pm 0.68

†Samples reflect averaging of values for eggs from the same clutch within each treatment (22, 30 and 12 clutches were included from NJ, SC and VA, respectively).

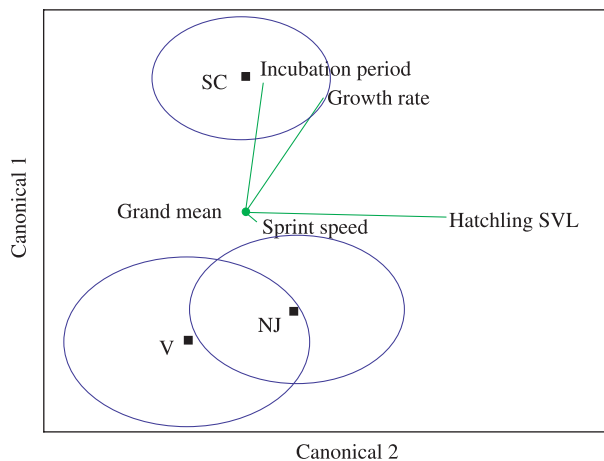


Fig. 1. A canonical plot showing variation in the mean values of traits among populations (NJ, VA and SC). Black squares represent the position of the centroid in canonical space (i.e. the grand mean of the vector of trait means). Spheres depict the 95% confidence intervals in multivariate space, as determined by MANOVA. Rays show original variables and their contribution to discrimination in the canonical space. Note, SC appears differentiated from VA and NJ along canonical axis 1, which mainly describes covariance between incubation period and growth rate. All populations overlap substantially along canonical axis 2, which mainly describes covariance between sprint speed and hatchling snout-vent length (SVL).

VA lizards cluster together and appear relatively distinct from the phenotypes of SC lizards (Fig. 1). Subsequent analysis of individual traits (ANOVA) exposed trends consistent with a previous experiment (Table 1; Oufiero & Angilletta 2006). Specifically, sizes at hatching were similar among populations ($F_{2,39} = 2.2$, $P = 0.12$), but lizards from NJ and VA hatched earlier than embryos from SC ($F_{2,39} = 14.1$, $P < 0.0001$). These observations imply that embryos from NJ and VA grew and developed faster than embryos from SC. Maximal sprint speed of hatchlings did not differ significantly among populations ($F_{2,39} = 0.07$, $P = 0.93$), but lizards from SC grew faster in length than lizards from NJ and VA ($F_{2,39} = 4.4$, $P = 0.02$).

Despite significant variation in incubation period and juvenile growth among populations (Fig. 1), CPCA did not reject the hypothesis of a common covariance structure among populations (Table 2). Three principal components of

Table 2. (a) Flury decomposition of the χ^2 statistic, using a step-up model-building approach (Phillips & Arnold 1999). At the highest level, we considered a model in which covariance matrices were the same for all populations. At the lowest level, we compared models in which the covariance matrices shared only one principal component (CPC) with a model in which the matrices have no components in common vs. being unrelated is tested

Model		χ^2	df	<i>P</i>	χ^2/df	AIC
Higher	Lower					
Equal	Proportional	0.442	2	0.8019	0.221	19.986
Proportional	CPC	15.513	6	0.0166	2.586	23.544
CPC	CPC (2)	0.098	2	0.9522	0.049	20.031
CPC (2)	CPC (1)	0.221	4	0.9943	0.055	23.933
CPC (1)	Unrelated	3.712	6	0.7156	0.619	31.712
Unrelated	–	–	–	–	–	40

(B) Eigenvectors for the best pooled covariance matrix

Trait	PC1 (26%)	PC2 (25%)	PC3 (34%)
Hatchling SVL	0.881	–0.105	0.248
Incubation Period	–0.103	–0.022	–0.719
Growth rate	–0.383	0.380	0.603
Sprint speed	0.257	0.919	–0.239

the CPCA describe 26%, 25% and 34% of the total variance (note: in CPCA, components are not necessarily extracted in order of the variance described; see (Phillips & Arnold 1999). The phenotypic space depicted by the three principal components contrasted SVL at hatching with juvenile growth rate (Fig. 2a,b) and incubation period (Fig. 2b,c). For example, PC1 describes an axis defined at one end by lizards that hatched at a large size but grew slowly after hatching, and at the other end by lizards that hatched at a small size but grew rapidly after hatching. Sprint speed loaded very strongly on PC2, but none of the other variables had a major influence on this axis. Finally, PC3 described an axis defined at one end by lizards that developed rapidly (short incubation period) as embryos and grew rapidly after hatching, and at the other end by lizards that developed slowly and grew slowly. Thus, the largest common component, PC3, revealed a relationship between incubation period and juvenile growth within populations that contrasted the relationship among populations (Fig. 3).

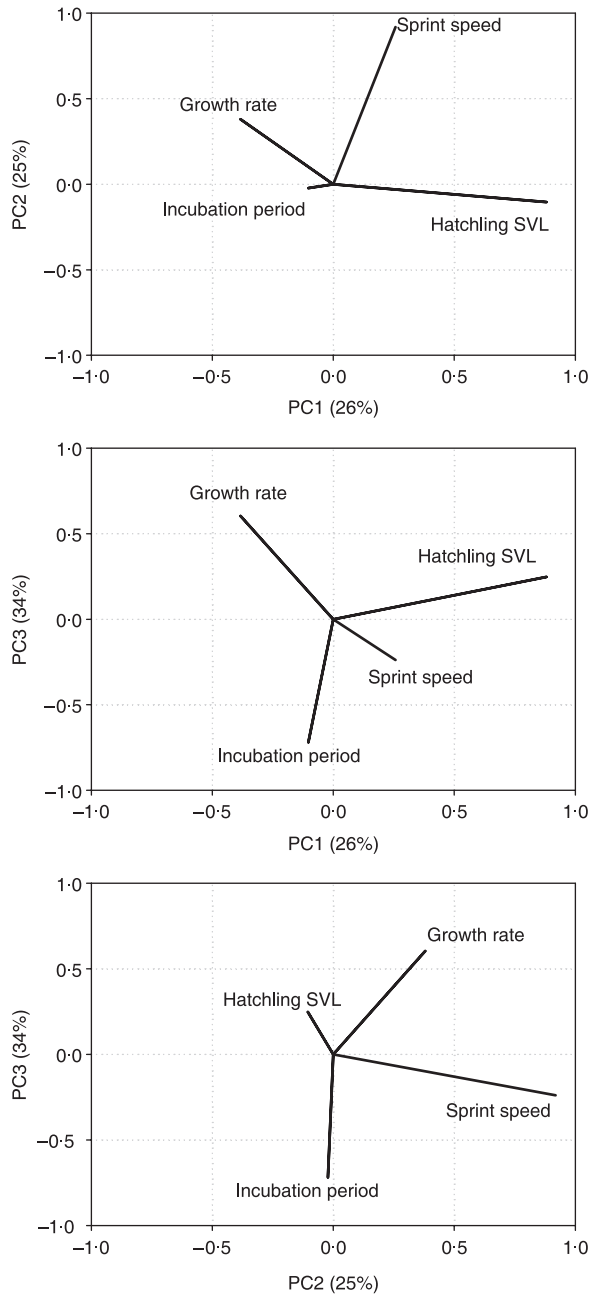


Fig. 2. Pairwise plots of principal components from a CPCA of the covariance matrix. Because principal components are orthogonal to one another, we can interpret each plot independently. Sprint speed was adjusted for body length prior to the analysis (see text for details). All populations share a common pattern of phenotypic covariation, even though mean values of several traits differ among populations (see Tables 1 and 2).

Discussion

The results of our experiment extend those of a previous experiment by Oufiero & Angilletta (2006), who concluded that countergradient variation in embryonic growth and development evolved independently in two clades of *S. undulatus*. In both experiments, fence lizards from cold environments grew and developed rapidly as embryos. Both experiments

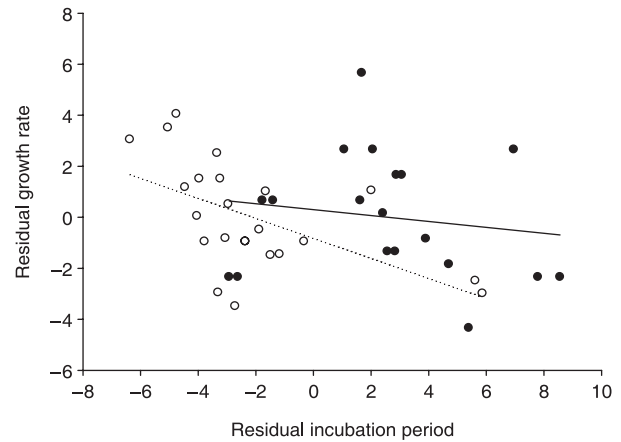


Fig. 3. The relationship between the residual incubation period of embryos and the residual growth rate of juveniles differed between lizards from northern and southern environments. The regression for northern populations (NJ and VA; open circles, dotted line) was significant ($F_{1,21} = 9.3$, $P = 0.002$), but that for SC (South; closed circles, solid line) was not ($F_{1,17} = 0.01$, $P = 0.9$). Note that a low residual incubation period translates to a relatively short developmental period; therefore, embryos that developed relatively rapidly grew relatively rapidly as juveniles. Data are multivariate residuals accounting for correlations between sprint speed and size at hatching.

included genotypes from VA, NJ and SC, and controlled for variation in egg size among populations. For countergradient variation to evolve by natural selection, rapid embryonic growth and development must impose costs that arise from one or more trade-offs (Gotthard, Nylin & Wiklund 2000; Angilletta *et al.* 2003). Our experiment was designed to test whether a trade-off between embryonic and juvenile performances could explain why genotypes from all environments do not grow and develop as rapidly as possible. We found significant differences in traits among populations, driven mainly by the prolonged embryonic development (i.e., a long incubation period) and rapid juvenile growth of SC lizards relative to VA and NJ lizards (Fig. 1). These differences among populations are consistent with the hypothesis that embryonic performance (e.g., incubation period) trades off with juvenile performance (e.g., growth rate).

Surprisingly, our analysis of phenotypic covariances within populations revealed two results that counter our hypothesis about trade-offs between embryonic and juvenile traits (see Fig. 2). Incubation period covaried negatively with juvenile growth rate. In other words, individuals that developed rapidly as embryos also grew rapidly during the first 60 days after hatching (Fig. 3). Consequently, although the phenotypic covariance among populations suggests a trade-off that could explain countergradient variation, the phenotypic covariances within populations do not. Furthermore, we could not reject the hypothesis that all populations shared a common covariance structure (see Table 2), even though the relationships between embryonic developmental rate and juvenile growth rate seemed to differ among populations (Fig. 3); specifically, lizards from NJ and VA exhibited a significant negative

relationship whereas lizards from SC show no significant relationship. Admittedly, low statistical power might account for the inability of the CPCA to reject a hypothesis of a common covariance structure (Phillips & Arnold 1999; Houle, Mezey & Galpern 2002; Mezey & Houle 2003), but direct inspection of the covariances supports the interpretation that no trade-off occurred at the individual level (Phillips & Arnold 1999; see Fig. 3 and Table 2b).

We can explain these contradictory results in several ways. First, countergradient variation might have resulted from genetic drift among populations, meaning we should expect no trade-off between embryonic and juvenile performances. While this possibility will always remain, we prefer to explore more interesting hypotheses before invoking chance as an explanation for phenotypic patterns. Second, countergradient variation might have evolved because of a trade-off between embryonic traits. For example, rapid embryonic growth might lead to a poor chance of survival to hatching. Our data fail to support this hypothesis because embryos from SC developed more slowly and were less likely to survive than were embryos from NJ and VA. Finally, our experiment might have been insufficiently designed to detect certain trade-offs between the embryonic and juvenile performances, even if these trade-offs do occur in natural environments. Of the three hypotheses, this last one seems most worthy of further discussion.

If natural selection caused the evolution of countergradient variation in *S. undulatus*, maximal rates of growth and development must not confer the greatest fitness in all natural environments (Arnott *et al.* 2006). Our findings suggest a trade-off between embryonic performances and either juvenile growth or locomotion cannot explain the evolution of countergradient variation in *S. undulatus*. Accordingly, an unidentified trade-off would have to constrain embryonic performance in nature. In free-ranging juveniles, rapid growth likely comes at the expense of a greater risk of predation, or some other source of mortality (Mangel & Stamps 2001). For example, Conover and colleagues (Billerbeck, Lankford & Conover 2001) showed that fast-growing genotypes of *Menidia menidia* fed more, swam slower, and hence suffered greater predation during staged encounters in the laboratory. Whether such trade-offs maintain countergradient variation in natural environments remains to be demonstrated for *M. menidia* or any other species. Given that rapid embryonic growth and development in *S. undulatus* entails depletion of yolk reserves (Storm & Angilletta 2007), hatchlings may emerge with less discretionary energy and greater metabolic demands. This need for intensive foraging would have imposed no risk in the artificial environment of our experiment, but would likely do so in a natural environment. Only a field experiment can definitively test this hypothesis for the maintenance of countergradient variation.

Even if we can identify a plausible trade-off, we must also explain why the inter-population covariance between embryonic and juvenile traits differed from the inter-individual covariance. The relatively rapid juvenile growth of SC lizards could have arisen from compensatory mechanisms triggered by slow embryonic growth. In our experiment, eggs were

incubated at the temperatures of nests in NJ and VA. Hatchlings were reared in a common environment, in which each lizard could behaviourally thermoregulate. Furthermore, energy during the embryonic stage was limited by egg size, but food after hatching was unlimited. Consequently, we did not equally constrain rates of embryonic and juvenile growth. Our incubation temperatures, although characteristic of NJ and VA, might have been relatively low for SC embryos. If so, we might have observed compensatory growth by SC lizards during the juvenile stage. Compensatory growth commonly follows periods of food deprivation in a diversity of ectotherms (Metcalf & Monaghan 2001), but may also follow periods of slow growth at low temperatures (Metcalf & Monaghan 2001; Hurst *et al.* 2005). Several complementary mechanisms could have produced compensatory growth, including greater feeding rates and more effective thermoregulation of juveniles (*sensu* Hertz, Huey & Stevenson 1993). The potential costs of such behaviours would not necessarily be manifested in the laboratory, which was free of predators, parasites, and other sources of mortality. Therefore, compensatory growth could have produced the negative covariation between embryonic and juvenile performances that we observed among populations (Fig. 1).

Field experiments (e.g., reciprocal transplants) will likely reveal more complex patterns of phenotypic covariation than those anticipated by classical theory, pointing the way towards better models of life-history evolution. In general, the rate at which empiricists have uncovered behavioural and physiological mechanisms underlying life-history strategies has outpaced the development of theory (Angilletta *et al.* 2003). For example, recent work on animals with complex life cycles suggests the phenotypes of adults depend on trade-offs that span multiple ontogenetic stages (De Block & Stoks 2005; Fischer *et al.* 2005; Ficetola & De Bernardi 2006; Stoks, De Block & Mcpeck 2006). Yet most models of life-history evolution compartmentalize rather than integrate these stages. This compartmentalization has likely limited our ability to predict how life histories evolve along environmental clines. Although we failed to find evidence for specific trade-offs between embryonic and juvenile performances, some remaining hypotheses for the evolution of countergradient variation concern the integration of phenotypes among life stages. Thus, broadening our focus from the independent evolution of traits to the evolutionary integration of traits should result in a more robust theory (Pigliucci 2003; Pigliucci & Preston 2004).

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