



Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae)

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The phenotypes of hatchling reptiles are known to be affected by the thermal environments they experience during incubation, but the evolutionary and ecological significance of this phenotypic plasticity remains unclear. Crucial issues include: (i) the *magnitude* of effects elicited by thermal regimes in natural nests (as opposed to constant-temperature incubation); (ii) the *persistence* of these effects during ontogeny; and (iii) the *consistency* of these effects across different test conditions (does the thermal regime during embryogenesis simply shift the hatchling's thermal optimum for performance, or actually modify overall performance ability regardless of temperature?). We examined these questions by incubating eggs of scincid lizards (*Bassiana duperreyi*) from montane southeastern Australia, under two fluctuating-temperature regimes that simulated 'cold' and 'hot' natural nests. These thermal regimes substantially modified hatchling morphology (mass, body length, tail length, and the relationship between these variables), locomotor performance (running speeds over distances of 25 cm and 1 m), anti-predator 'tactics' and survival rates. The differences in locomotor performance persisted throughout the 20 weeks of our experiment. Lizards that emerged after 'hot' incubation were faster runners than their 'cold'-incubated siblings under all thermal conditions that we tested. Thus, incubation temperatures modified overall locomotor ability, with only a minor effect on the set-point for optimum performance. The magnitude, persistence and consistency of these incubation-induced phenotypic modifications suggest that they may play an important role in evolutionary and ecological processes within lizard populations.

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INTRODUCTION

The phenotype of an organism is not solely the result of its genotype. Environmental variables can also exert a powerful effect on phenotypic traits, and recent years have seen a growing appreciation of the potential evolutionary significance of this kind of phenotypic plasticity (e.g. Roff, 1992; Stearns, 1992; Rollo, 1995). For example, traits will not evolve (i.e. show longterm directional change) even under intense natural selection, if the variance in those traits is engendered by the local environment rather than genetic factors. Given the widespread occurrence of phenotypic plasticity, and its potential influence on the ways in which selective pressures translate into evolutionary shifts, we need a clearer understanding of the nature, magnitude and persistence of environmentally-induced modifications to the phenotype.

Phenotypic plasticity can operate at any stage of an organism's life history, but its influence during early ontogeny may be of particular importance. Embryogenesis is a crucial stage in the life-history of all organisms, but the potential impact of environmental factors is greatest in oviparous (egg-laying) species, where a large proportion of development occurs outside the mother's body. In oviparous reptiles, the incubation conditions experienced during embryogenesis have been shown to influence a wide variety of hatchling traits including size (Gutzke & Packard, 1987; Beauchat, 1988; Whitehead & Seymour, 1990; Seymour, Geiser & Bradford, 1991; Van Damme *et al.*, 1992), shape (Fox, 1948; Osgood, 1978; Burger, 1990), colour (Vinegar, 1974; Ewart, 1979; Deeming & Ferguson, 1989, 1991), gender (Bull, 1980; Ferguson & Joanen, 1982; Brooks *et al.*, 1991), and a suite of behavioural and performance characteristics (e.g. Burger, 1989, 1990, 1991; Van Damme *et al.*, 1992; Janzen, 1993b). Reptiles thus offer useful model systems in which to investigate the biological significance of phenotypic plasticity.

Although the influence of incubation temperature on hatchling reptile morphology is well-documented, the ecological and evolutionary significance of these effects remains obscure. In order to evaluate this issue, we need information on:

(1) The *magnitude* of modifications to the phenotype induced by thermal regimes in natural nests. Thus, we need to measure actual nest temperatures, and incubate eggs under conditions that encompass the range of natural variation (e.g. among nests within a population, or among seasons, or among years). Most previous

research on environmentally-induced phenotypic variation has not been performed under biologically realistic conditions. For example, studies of incubation effects typically involve incubation at constant, or at best two-step temperature functions (e.g. Paukstis, Gutzke & Packard, 1984; Miller, Packard & Packard, 1987; Burger, 1989, 1990, 1991; Van Damme *et al.*, 1992; Dmi'el *et al.*, 1994; Spotila *et al.*, 1994), whereas natural nest temperatures typically undergo sinusoidal diel fluctuations, often with a large variance about the mean (Packard & Packard, 1988). Because both the mean and the variance of incubation temperature influence hatchling phenotypes, results from constant-temperature incubation may be difficult to extrapolate to the field (Shine & Harlow, 1996; Shine, Elphick & Harlow, 1997).

(2) The *persistence* of incubation-induced modifications during the ontogeny of the organism. Most previous studies have relied upon measures taken on hatchlings, and many such effects may well prove to be transient (e.g. Phillips *et al.*, 1990; Shine & Harlow, 1993; Shine, 1995; but see Burger, 1989, 1990, 1991). The biological significance of such effects will depend on the time-scale of events in the organism's life-history: for example, even a brief effect may be important if it spans a period of high and selective mortality. Relatively short-lived, early-maturing organisms are likely to offer the best (i.e. logistically feasible) study systems in this respect, rather than the very long-lived animals (such as turtles and crocodilians) that have attracted most previous work.

(3) The *consistency* of incubation-induced modifications when measured under different test conditions. Measures of locomotor ability are strongly influenced by test conditions (especially, ambient temperature: e.g. Kaufmann & Bennett, 1989; Mautz, Daniels & Bennett, 1992). Although several studies have documented changes in locomotor performance (running or swimming speeds) due to incubation temperatures, all have tested offspring at only one temperature (e.g. Burger, 1989; Van Damme *et al.*, 1992; Janzen, 1993b; Shine & Harlow, 1993; Shine, 1995). One plausible way in which incubation temperatures might affect organismal performance is by means of modifying the animal's thermal 'set-point' for optimal performance (e.g. Burger, 1989); that is, overall mean performance levels are unaffected, but hatchlings from different incubation regimes have their peak performance under different test temperatures. If this is the case, the fitness consequences of incubation-induced changes are likely to be less significant than they would be if the overall performance ability of the organism (regardless of test temperature) was the trait that was modified.

Our study was designed to address these questions, by measuring the effects of 'natural' levels of among-nest variation in incubation temperatures on the morphology (body size and shape) and locomotor performance of lizards in their first 5 months of life. We examined hatchling locomotor performance at a range of body temperatures, to determine whether incubation temperatures shifted thermal optima for performance, or modified overall performance ability regardless of temperature.

MATERIAL AND METHODS

Egg collection and incubation

The montane scincid lizard *Bassiana duperreyi* (= '*Leiolopisma trilineatum*' in older literature) is a medium-sized (to 80mm snout-vent length) oviparous diurnal skink

that is distributed throughout much of sub-alpine southeastern Australia (Cogger, 1992). Females lay a single clutch of three to eight eggs in midsummer (December–January: Pengilley, 1972; Shine, 1983). Because oviposition among *B. duperreyi* is highly synchronous (Pengilley, 1972; Shine, 1983, 1995; Shine & Harlow, 1996), we could time our fieldwork to coincide with egg-laying. Over the summer of 1994–95, we collected recently-oviposited eggs from four communal nest sites at Coree Flats and Piccadilly Circus (elevation 1050m and 1246m respectively) in the Brindabella Ranges, 40km west of Canberra in the Australian Capital Territory. These eggs, plus 13 gravid *B. duperreyi* collected from the same sites, were transported to the University of Sydney. Upon arrival, the eggs were individually weighed (± 0.001 g) on a Sartorius top-loading balance. They were then transferred to individually labelled 64 ml glass jars containing vermiculite (water potential = -200 kPa), and sealed with plastic cling wrap to prevent moisture loss throughout incubation.

All of the ovigerous females oviposited within 12 days of capture. Once eggs had been laid, they were treated in the same way as the field-laid eggs. Field-laid eggs from each of the nests, and each clutch of laboratory-laid eggs, were divided equally between two incubation treatments to minimize any maternal or nest effects (i.e. 'split-clutch' design). Clayson 10-step programmable incubators were set to mimic the thermal regimes characteristic of natural nests (Shine & Harlow, 1996). The 'cold' incubator was set to approximate cool nests (sinusoidal daily curve, $20 \pm 4^\circ\text{C}$), whereas the 'hot' incubator was programmed to fluctuate around a higher mean temperature ($27 \pm 4^\circ\text{C}$). Both incubators were checked twice daily for hatchling lizards. Any newly emerged hatchlings were weighed and measured (see below), before being removed to a temperature-controlled room in the Native Animal House.

Husbandry

We maintained lizards from each treatment group under identical conditions once they left the incubators. Hatchlings were housed individually in plastic cages ($22 \times 13 \times 7$ cm), containing a plywood shelter at the hot end of the cage (see below), and a water dish at the cooler end. A substrate of commercial potting mix covered the floor of the cages. Hatchlings were fed mealworms (*Tenebrio* larvae) twice weekly, and water was provided *ad libitum*. Air temperature within the room was kept at $20 \pm 1^\circ\text{C}$, but one end of each cage was placed over a heating cable to allow captives the opportunity for behavioural thermoregulation. This design produced a thermal gradient over the length of the cage of $23 \pm 0.4^\circ\text{C}$ at the cooler end, to $34 \pm 1.0^\circ\text{C}$ at the hot end. The heating cable and room lights were controlled by electronic timers that were synchronized to create a 10h light (warm) : 14h dark (cool) cycle. During the day when lights and heating racks were operational, room temperature rose to an ambient of $23 \pm 1^\circ\text{C}$. Overnight, cage substrate temperatures fell to $20 \pm 1^\circ\text{C}$.

Morphology

Hatchling lizards were weighed on a Sartorius top-loading balance (± 0.01 g), and snout–vent length (hereafter, SVL) and total body length were also recorded

(± 0.5 mm). From these measurements, we calculated tail length (total length minus SVL). We recorded offspring mass again when lizards were a week old, and recorded mass, SVL, and total length at 2, 4, 6, 14 and 20 weeks of age.

Locomotor performance

We measured the running speeds of *Bassiana duperreyi* offspring over a range of body temperatures, when the lizards were 2, 6, 14 and 20 weeks of age. We used temperature-controlled rooms to maintain lizard body temperatures at 15, 20, 25 or 30°C ($\pm 1^\circ\text{C}$). Lizards were transported to the appropriate rooms in individual holding jars and left to acclimate undisturbed for at least 30 minutes prior to running. The raceways were similarly acclimated to the required temperature. Following acclimation, lizards were transferred to the holding area of the raceway before being released and allowed to run the 1-m distance. If necessary, we used an artist's paintbrush to stimulate them to keep running. Speeds (m/s) were determined with an infra-red timing device, using photocells at 25 cm intervals along the runway to record the cumulative time taken for lizards to cross each successive infra-red beam.

We tested each age-group of lizards at the four temperatures over four consecutive days. The order of temperatures at which lizards were run was randomized to control for any habituation effects. Lizards were rested for at least 10 minutes between each of three trials they underwent at the designated temperature for that day. We calculated mean running speed over 1 m, and mean 'burst speed' (defined as the fastest speed recorded over a 25 cm distance), for each lizard at each body temperature. We also noted and recorded one distinctive behaviour during the running trials of *B. duperreyi* offspring. Some lizards stopped midway through a trial, reversed direction, and then scampered past the paintbrush in the direction from whence they came, frantically wriggling the vertically-raised tail. This 'raised tail wag' has been reported previously in *B. duperreyi* (Shine, 1995), and also occurs in other scincid species (*Lampropholis guichenoti*—F. Qualls, pers. comm.; *Eulamprus tympanum* hatchlings—P. Doughty, pers. comm.).

Statistical analyses

We checked all data for normality (Shapiro–Wilk test) and homoscedasticity (Bartlett's test); no significant deviations from these assumptions were evident in ln-transformed data. All analyses were tested for statistical significance at the $P < 0.05$ level. Since our interest lay not only in whether differences were apparent, but also in how long any such differences persisted, we looked for effects of incubation in each age cohort separately using one-factor analysis of variance (ANOVA), with incubation treatment as the factor. Because these tests are based on data from the same individuals at different ages, they are not statistically independent. For this reason, we applied sequential Bonferroni corrections (Rice, 1989) to all tests on each variable.

One-factor analysis of covariance (ANCOVA) was employed to investigate the possible influence of incubation temperature on relative body mass and relative tail length of offspring in each age cohort (with incubation treatment as the factor, SVL

as the covariate, and mass or tail length as the respective dependent variables). Where slopes were not significantly different ($P > 0.05$), interaction terms were deleted and the analyses recalculated to investigate possible differences in elevation (intercept) arising from the experimental treatment (Sokal & Rohlf, 1981). Differences in locomotor performance between lizards from the 'hot' and 'cold' incubation treatments were examined in each age cohort using one-factor analysis of variance (ANOVA), with incubation treatment as the factor and running speeds over one metre and 25 cm as the dependent variables. We also performed one-factor analysis of covariance (ANCOVA), with incubation treatment as the factor, mass, snout-vent length and tail length as the respective covariates, and running speed over 1 m as the dependent variable, to determine whether offspring size accounted for any of the observed variation in locomotor performance. Additionally, simple regression analyses of running speeds *versus* offspring body shape (i.e. residual scores from the general linear regressions of mass or tail length *versus* SVL) were performed to examine possible effects of body shape on locomotion.

We examined differences between the 'hot' and 'cold' incubation treatments in the frequency of 'raised tail wag' behaviour using chi-square analyses. We also pooled the data for 'hot' and 'cold' lizards and tested for differences in the frequency of this behaviour at lower temperatures (i.e. from 15 and 20°C combined) *versus* at higher temperatures (i.e. 25 and 30°C combined) using χ^2 analysis. All of our analyses were tested for statistical significance at the $P < 0.05$ level.

RESULTS

Egg mass

The split-clutch design, whereby eggs from each clutch were randomly allocated to 'hot' *versus* 'cold' treatments, was designed to remove any confounding differences in mean mass of eggs assigned to each incubation treatment. In this respect, we were successful (one-factor ANOVA with incubation treatment as the factor and egg mass as the dependent variable: $F = 0.08$, $df = 1, 137$, $P = 0.77$; based only on laboratory-laid eggs: $F = 0.09$, $df = 1, 35$, $P = 0.77$).

Egg mortality

Mortality of eggs was not influenced by incubation temperature. Although six eggs (8.7%) from the 'cold' incubator failed to hatch *versus* three (4.3%) from the 'hot' incubator, this difference was not statistically significant ($\chi^2 = 0.98$, $df = 1$, $P = 0.32$).

Incubation period

Incubation temperatures profoundly affected developmental rates. Eggs from the 'cold' incubator had a significantly longer incubation period (mean = 79.1 ± 6.5 days) than eggs from the 'hot' incubator (mean = 30.9 ± 1.5 days) (one-factor ANOVA

with incubation period as the factor: $F=3649.7$, $df=1,137$, $P<0.0001$; using only laboratory-laid eggs, means = 79.9 ± 4.9 versus 32.0 ± 1.3 days: $F=1878.0$, $df=1,37$, $P<0.0001$).

Offspring mortality

Overall mortality for the 6-month study was high. Of the 139 lizards that hatched in the laboratory, only 24 (17%) were alive at the completion of the study. The surviving lizards included 16 of the 70 hot-incubated lizards (23%) and eight of the 69 cold-incubated lizards (12%). The survival rate of lizards from the 'hot' incubation treatment over the 24 week sampling period was significantly higher than for lizards incubated at the 'cold' temperature (Kaplan–Meier estimate with Mantel–Cox logrank test: $\chi^2=19.39$, $df=1$, $P<0.0001$). Interestingly, our data also suggest that offspring survival was linked to size at hatching. Larger offspring showed higher survival: mean mass at hatching was significantly lower for lizards that died during the study than for those that survived (pooled 'hot' and 'cold': $t=2.95$, $df=137$, $P<0.004$); this trend was stronger for cold-incubated lizards than their hot-incubated siblings ('cold' only: $t=2.27$, $df=67$, $P<0.03$; 'hot' only: $t=1.65$, $df=68$, $P=0.10$).

Offspring body size

Mean mass of *B. duperreyi* offspring varied between incubation treatments, with the hot-incubated lizards hatching heavier than those from the 'cold' incubator (Table 1). Given the equivalence in mean egg sizes for the two treatments (see above), the difference in hatchling mass, though not statistically significant, suggests that a given-size egg produced a larger hatchling under 'hot' incubation. To examine this possibility, we analysed hatchling mass relative to egg mass. As expected, hot-incubated hatchlings were significantly heavier than cold-incubated lizards that emerged from eggs of identical original mass (one-factor ANCOVA with incubation treatment as the factor, egg mass as the covariate and hatchling mass as the dependent variable; homogeneity of slopes, $F=0.65$, $df=1,135$, $P=0.42$; intercepts test, $F=4.34$, $df=1,136$, $P<0.04$). These differences in offspring mass persisted for some time (Table 1).

Between-treatment differences in offspring snout-vent length were minor and inconsistent, but incubation temperature had a strong effect on tail length at hatching. Hot-incubated lizards had significantly longer tails than did cold-incubated hatchlings for at least the first 6 weeks of life (Table 1).

Offspring body shape

At the same SVL, lizards from the 'hot' incubation treatment were heavier, and had longer tails, than lizards from the 'cold' incubator (Table 2). These effects persisted for at least 4 weeks (Table 2).

TABLE 1. Mean mass, SVL and tail length of *Bassiana duperreyi* offspring from each age cohort, based on raw data (\pm SD). This table also shows the results of statistical analyses on the effects of incubation temperature on morphological variables of lizards from each age cohort, using ln-transformed data (one-factor ANOVA with incubation treatment as the factor). Note that significance levels have been corrected for multiple tests using the sequential Bonferroni technique, applied within each group of tests

Variable	Age (weeks)	'Hot'		'Cold'		Statistical test: Effect of incubation temperature
		Mean (SD)	Count	Mean (SD)	Count	
Mass (g)	0	0.28 (0.03)	70	0.27 (0.03)	69	$F=4.24$, $df=1,137$, $P=0.20$
	1	0.28 (0.03)	70	0.26 (0.03)	68	$F=22.40$, $df=1,136$, $P<0.001$
	2	0.31 (0.05)	68	0.26 (0.04)	62	$F=47.63$, $df=1,128$, $P<0.001$
	4	0.37 (0.06)	68	0.28 (0.07)	54	$F=49.46$, $df=1,120$, $P<0.001$
	6	0.43 (0.07)	61	0.42 (0.08)	34	$F=0.91$, $df=1,93$, $P=0.34$
	14	0.64 (0.13)	37	0.68 (0.11)	25	$F=1.69$, $df=1,60$, $P=0.20$
	20	0.80 (0.15)	23	0.81 (0.11)	13	$F=0.09$, $df=1,34$, $P=0.77$
Snout-vent length (mm)	24	0.88 (0.15)	16	0.81 (0.13)	8	$F=1.10$, $df=1,22$, $P=0.31$
	0	24.71 (1.09)	70	25.37 (0.97)	69	$F=13.88$, $df=1,137$, $P<0.002$
	2	26.32 (1.22)	68	26.16 (1.02)	62	$F=0.59$, $df=1,128$, $P=0.44$
	4	27.86 (1.13)	68	26.66 (1.12)	54	$F=33.86$, $df=1,120$, $P<0.0007$
	6	28.84 (1.31)	61	28.16 (1.36)	34	$F=5.79$, $df=1,93$, $P=0.10$
	14	31.05 (1.96)	37	31.82 (1.35)	25	$F=3.02$, $df=1,60$, $P=0.09$
	20	33.74 (1.88)	23	33.35 (1.71)	13	$F=0.34$, $df=1,34$, $P=0.56$
Tail length (mm)	24	34.94 (2.29)	16	34.56 (1.88)	8	$F=0.12$, $df=1,22$, $P=0.73$
	0	30.66 (1.56)	70	28.33 (1.93)	69	$F=60.87$, $df=1,137$, $P<0.001$
	2	32.35 (1.87)	64	30.45 (2.05)	62	$F=24.42$, $df=1,124$, $P<0.001$
	4	35.23 (2.39)	64	31.25 (2.48)	54	$F=81.16$, $df=1,116$, $P<0.001$
	6	36.97 (2.43)	57	33.85 (3.70)	33	$F=24.82$, $df=1,88$, $P<0.001$
	14	41.05 (3.19)	33	41.06 (3.90)	24	$F=0.002$, $df=1,55$, $P=0.97$
	20	44.21 (3.97)	20	42.94 (3.81)	13	$F=1.35$, $df=1,31$, $P=0.25$
	24	45.10 (4.42)	16	44.56 (4.34)	8	$F=0.008$, $df=1,20$, $P=0.42$

Locomotor performance

Incubation temperature strongly influenced offspring locomotor performance. Hot-incubated *Bassiana duperreyi* ran significantly faster than cold-incubated offspring at body temperatures of 15, 20, 25 and 30°C when measured at 2, 6, 14 and 20 weeks of age (Figs 1 and 2). This was a remarkably consistent result: hot-incubated offspring averaged faster than those from the 'cold' incubator in every trial, and the same pattern was evident at both distances tested (i.e. over 1 m and over 0.25 m).

Relationship of running speeds to body size and shape

To explore the reasons for the consistent differences in offspring running speeds between incubation treatments, we performed one-factor analyses of covariance (ANCOVA) with mass, SVL and tail length as the respective covariates to see if differences in offspring body size accounted for any of the differences in locomotor performance over 1 m. We chose these morphological variables because earlier tests showed that they were influenced by incubation temperature (see above). Our analyses revealed that although offspring size influenced running speed (i.e. heavier and longer lizards ran faster), the relationship between offspring size and running speed was also significantly affected by incubation treatment, independent of any

TABLE 2. This table shows the results of statistical analyses on the effects of incubation temperature on body proportions of *Bassiana duperreyi* offspring. The traits tested are (a) mass relative to SVL, and (b) tail length relative to SVL. The table compares 'hot' and 'cold' treatment groups at various ages, using ln-transformed data (one-factor ANCOVA with incubation treatment as the factor, SVL as the covariate and offspring mass or tail length as the dependent variables). Note, an intercepts test was not carried out if slopes were heterogeneous ($P < 0.05$). Note that significance levels have been corrected for multiple tests using the sequential Bonferroni technique, applied within each group of tests

Body proportion	Age (weeks)	Statistical test: effect of incubation temperature	
		Homogeneity of slopes test	Intercepts test
mass relative to SVL	0	$F=0.78, df=1,135, P=0.38$	$F=22.21, df=1,136, P<0.001$
	2	$F=2.23, df=1,126, P=0.14$	$F=100.37, df=1,127, P<0.001$
	4	$F=0.09, df=1,118, P=0.77$	$F=12.48, df=1,119, P<0.003$
	6	$F=0.72, df=1,91, P=0.72$	$F=2.66, df=1,92, P=0.11$
	14	$F=0.37, df=1,58, P=0.55$	$F=0.24, df=1,59, P=0.63$
	20	$F=0.98, df=1,32, P=0.33$	$F=2.81, df=1,33, P=0.10$
tail length relative to SVL	24	$F=0.02, df=1,20, P=0.88$	$F=2.41, df=1,21, P=0.14$
	0	$F=0.30, df=1,135, P=0.58$	$F=88.86, df=1,136, P<0.001$
	2	$F=1.02, df=1,122, P=0.31$	$F=24.00, df=1,123, P<0.001$
	4	$F=0.66, df=1,114, P=0.42$	$F=34.57, df=1,115, P<0.001$
	6	$F=8.42, df=1,86, P<0.005$	
	14	$F=5.32, df=1,53, P<0.03$	
	20	$F=0.01, df=1,29, P=0.91$	$F=1.70, df=1,30, P=0.20$
	24	$F=0.94, df=1,20, P=0.34$	$F=0.09, df=1,21, P=0.76$

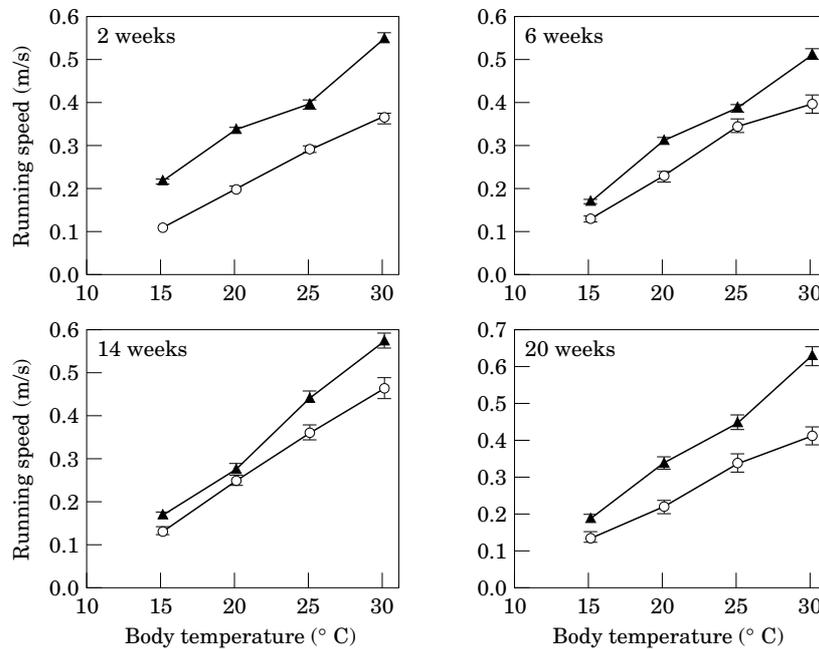


Figure 1. Mean running speeds of lizards (*Bassiana duperreyi*) at four different body temperatures, over a distance of 1 m. The four graphs show data for the same lizards at different ages. Data (means \pm 1 SE) are presented separately for lizards from 'cold' incubation (\circ) and their siblings from 'hot' incubation (\blacktriangle) (see text for definitions).

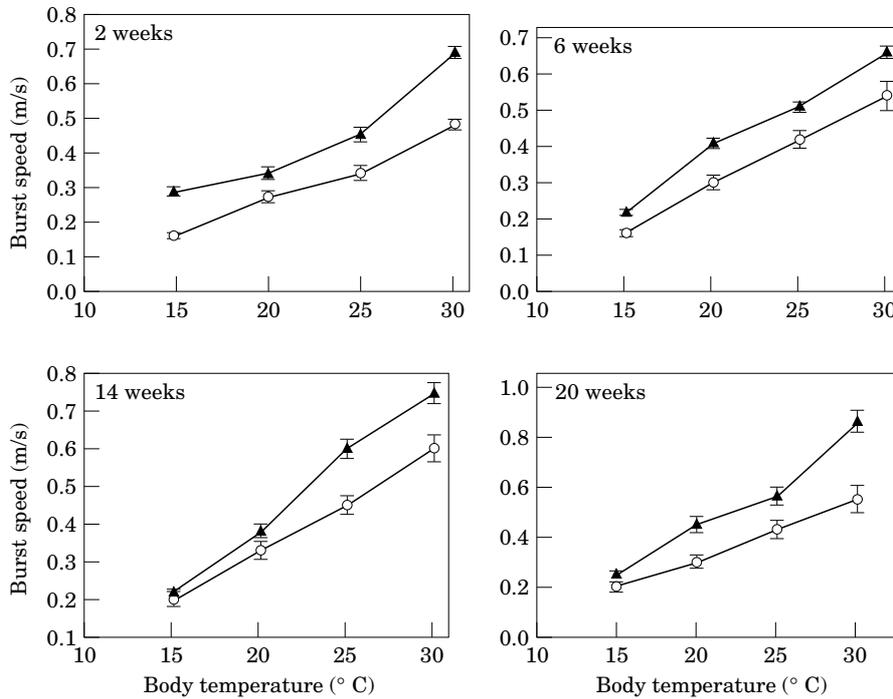


Figure 2. Mean running speeds of lizards (*Bassiana duperreyi*) at four different body temperatures, over a distance of 25 cm. The four graphs show data for the same lizards at different ages. Data (means \pm 1 SE) are presented separately for lizards from 'cold' incubation (○) and their siblings from 'hot' incubation (▲) (see text for definitions).

body-size effects. That is, differences in running speeds between incubation treatments were significant even after the effects of body size were removed (Table 3a,b,c). Hot-incubated lizards ran faster than same-sized lizards from the 'cold' incubation treatment at all body temperatures tested, over the duration of this study.

The other morphological traits modified by incubation temperatures involve body shape. However, regression analyses of running speeds *versus* body shape (i.e. residuals of mass and tail length relative to snout-vent length) explained very little of the variation in offspring running speeds between incubation treatments ($r^2 < 0.05$ in all cases). Hence, the effects of incubation temperature on body size and shape cannot explain the large and consistent differences we measured in running speeds throughout the study.

Running speeds at different body temperatures

The faster running speeds of 'hot'-incubated lizards were evident at all of the thermal conditions under which we tested the young lizards (Figs 1 and 2). Nonetheless, the disparity in running speeds over 1 m between 'hot'- and 'cold'-incubated lizards differed among test temperatures; the superiority of 'hot'-incubated lizards was greater at 30°C than at lower test temperatures (Figs 1 and 2). Our statistical analyses thus revealed a significant interaction between incubation condition

TABLE 3. These tables show the results of statistical analyses on the effects of incubation temperature on mean running speed (m/s) of *Bassiana duperreyi* offspring over 1 m relative to body size, using ln-transformed data (one-factor ANCOVA with incubation treatment as the factor, a body size variable as the covariate, and running speed as the independent variable). Note that significance levels have been corrected for multiple tests using the sequential Bonferroni technique, applied within each group of tests

Age (weeks)	Body Temp (°C)	Statistical test: Effect of incubation temperature	
		Homogeneity of slopes test	Intercepts test
(a) Running speed relative to body mass			
2	15	$F=3.79, df=1,120, P=0.06$	$F=322.68, df=1,121, P<0.001$
	20	$F=3.03, df=1,1200, P=0.08$	$F=151.93, df=1,121, P<0.001$
	25	$F=1.25, df=1,121, P=0.27$	$F=31.60, df=1,122, P<0.001$
	30	$F=0.11, df=1,120, P=0.75$	$F=12.56, df=1,121, P<0.001$
6	15	$F=3.42, df=1,83, P=0.32$	$F=25.39, df=1,84, P<0.001$
	20	$F=3.26, df=1,78, P=0.08$	$F=51.63, df=1,79, P<0.001$
	25	$F=0.75, df=1,83, P=0.39$	$F=8.51, df=1,84, P<0.001$
	30	$F=1.87, df=1,79, P=0.18$	$F=33.61, df=1,80, P<0.001$
14	15	$F=0.001, df=1,54, P=0.97$	$F=19.98, df=1,55, P<0.001$
	20	$F=0.33, df=1,56, P=0.57$	$F=7.27, df=1,56, P<0.009$
	25	$F=0.03, df=1,56, P=0.86$	$F=24.81, df=1,57, P<0.001$
	30	$F=0.08, df=1,52, P=0.78$	$F=21.64, df=1,52, P<0.001$
20	15	$F=0.08, df=1,28, P=0.77$	$F=14.96, df=1,29, P<0.002$
	20	$F=0.17, df=1,28, P=0.68$	$F=18.97, df=1,29, P<0.0006$
	25	$F=0.03, df=1,28, P=0.86$	$F=11.75, df=1,29, P<0.002$
	30	$F=1.45, df=1,28, P=0.24$	$F=27.14, df=1,29, P<0.001$
(b) Running speed relative to snout-vent length. Note that an intercepts test was not carried out if the slopes were heterogeneous ($P<0.05$)			
2	15	$F=0.58, df=1,120, P=0.45$	$F=519.41, df=1,121, P<0.001$
	20	$F=0.16, df=1,120, P=0.69$	$F=267.45, df=1,121, P<0.001$
	25	$F=0.01, df=1,121, P=0.95$	$F=85.30, df=1,122, P<0.001$
	30	$F=28, df=1,120, P=0.60$	$F=130.29, df=1,121, P<0.001$
6	15	$F=3.25, df=1,83, P=0.08$	$F=19.11, df=1,84, P<0.0002$
	20	$F=6.02, df=1,78, P<0.02$	NA
	25	$F=2.99, df=1,83, P=0.09$	$F=5.40, df=1,84, P<0.02$
	30	$F=6.53, df=1,79, P<0.01$	NA
14	15	$F=0.65, df=1,54, P=0.42$	$F=19.38, df=1,55, P<0.001$
	20	$F=0.08, df=1,56, P=0.78$	$F=8.45, df=1,57, P<0.005$
	25	$F=0.54, df=1,56, P=0.47$	$F=26.91, df=1,57, P<0.001$
	30	$F=1.30, df=1,52, P=0.26$	$F=22.71, df=1,53, P<0.001$
20	15	$F=0.18, df=1,28, P=0.67$	$F=12.91, df=1,29, P<0.002$
	20	$F=0.04, df=1,28, P=0.85$	$F=19.56, df=1,29, P<0.001$
	25	$F=0.01, df=1,28, P=0.92$	$F=11.95, df=1,29, P<0.002$
	30	$F=1.43, df=1,28, P=0.24$	$F=29.15, df=1,29, P<0.001$

continued

and test temperature for running speeds over 1 m (at 2 wk, interaction $F_{3,489} = 11.35, P<0.0001$; at 6 wk, interaction $F_{3,321} = 5.30, P<0.0015$; at 14 wk, interaction $F_{3,226} = 4.15, P<0.007$; at 20 wk, interaction $F_{3,120} = 4.93, P<0.003$) but not for speeds over 0.25m (at 2 wk, interaction $F_{3,454} = 1.84, P=0.14$; at 6 wk, interaction $F_{3,296} = 0.89, P=0.44$; at 14 wk, interaction $F_{3,209} = 0.62, P=0.60$; at 20 wk, interaction $F_{3,111} = 0.97, P=0.41$). Thus, our data show that (i) the thermal conditions experienced during incubation affected the overall locomotor ability of the young lizards, regardless of the temperature at which they were tested, and that (ii) incubation conditions also shifted the relationship between temperature and performance (at

TABLE 3. (continued)

Age (weeks)	Body Temp (°C)	Statistical test: Effect of incubation temperature	
		Homogeneity of slopes test	Intercepts test
(c) Running speed relative to tail length. Note that an intercepts test was not carried out if the slopes were heterogeneous ($P < 0.05$)			
2	15	$F = 4.78$, $df = 1,120$, $P < 0.03$	NA
	20	$F = 1.9$, $df = 1,120$, $P = 0.20$	$F = 258.69$, $df = 1,121$, $P < 0.001$
	25	$F = 0.97$, $df = 1,121$, $P = 0.33$	$F = 74.08$, $df = 1,122$, $P < 0.001$
	30	$F = 1.23$, $df = 1,120$, $P = 0.27$	$F = 120.3$, $df = 1,121$, $P < 0.001$
6	15	$F = 0.27$, $df = 1,83$, $P = 0.60$	$F = 17.09$, $df = 1,84$, $P < 0.001$
	20	$F = 0.01$, $df = 1,78$, $P = 0.95$	$F = 36.17$, $df = 1,79$, $P < 0.001$
	25	$F = 1.31$, $df = 1,83$, $P = 0.26$	$F = 4.39$, $df = 1,84$, $P < 0.04$
	30	$F = 0.88$, $df = 1,79$, $P = 0.35$	$F = 23.56$, $df = 1,80$, $P < 0.001$
14	15	$F = 0.47$, $df = 1,54$, $P = 0.50$	$F = 15.32$, $df = 1,55$, $P < 0.003$
	20	$F = 0.04$, $df = 1,56$, $P = 0.85$	$F = 4.76$, $df = 1,57$, $P < 0.03$
	25	$F = 0.12$, $df = 1,56$, $P = 0.74$	$F = 22.43$, $df = 1,57$, $P < 0.001$
	30	$F = 0.05$, $df = 1,52$, $P = 0.83$	$F = 19.59$, $df = 1,53$, $P < 0.001$
20	15	$F = 0.70$, $df = 1,28$, $P = 0.41$	$F = 14.30$, $df = 1,29$, $P < 0.001$
	20	$F = 0.85$, $df = 1,28$, $P = 0.36$	$F = 20.54$, $df = 1,29$, $P < 0.001$
	25	$F = 0.01$, $df = 1,28$, $P = 0.92$	$F = 15.03$, $df = 1,29$, $P < 0.001$
	30	$F = 2.03$, $df = 1,28$, $P = 0.17$	$F = 29.59$, $df = 1,29$, $P < 0.001$

least for running speeds over 1 m). It is important to note that the second of these effects is relatively minor. If acclimation effects of this kind were very strong, we would expect to see that ‘cold’-incubated lizards would be slower than their ‘hot’-incubated siblings at some test temperatures, but faster at others. Instead, the ‘hot’-incubated lizards were always the faster runners (Figs 1 and 2). The magnitude of the difference in running speeds varied only slightly among test temperatures, and was not detectable when speeds were measured over 0.25 m. Thus, our results demonstrate a strong and relatively consistent performance superiority in the ‘hot’-incubated lizards.

Anti-predator behaviour

The frequency of ‘raised tail wag’ (RTW) behaviour was related to lizard body temperature, age, and in some cases the temperature at which lizards were incubated. This behaviour occurred at a significantly higher frequency during trials conducted at lower body temperatures (i.e. 15 and 20°C *versus* 25 and 30°C) ($\chi^2 = 26.26$, $df = 1$, $P < 0.001$), and at younger ages. Almost half of all the RTW behaviour that we observed throughout the study occurred in the first performance trials, when lizards were two weeks old (pooled ‘hot’ and ‘cold’ RTW = 47%). Additionally, at 2 weeks of age hot-incubated lizards displayed this behaviour at a significantly higher frequency than did lizards from the ‘cold’ incubation treatment ($\chi^2 = 6.81$, $df = 1$, $P < 0.009$). By 6 weeks of age, this difference was no longer evident, and it did not reappear for the duration of the study.

DISCUSSION

Our data allow us to address the three issues that we posed in the introduction to this paper: the magnitude, persistence and consistency of incubation-induced changes to the phenotypes of young lizards.

Magnitude of incubation-induced changes

Our experimental treatments simulated the thermal regimes recorded in natural nests of *Bassiana duperreyi*; indeed, the same nest-sites from which we removed eggs during this study. Thus, our laboratory simulations accurately reproduced thermal (but possibly not hydric) conditions within natural nests. In keeping with this conclusion, eggs laid in natural nests in our study area in December 1994 (at about the time we collected our 'experimental' eggs) hatched in the field midway between the times of hatching of our 'cold' and 'hot' nest simulations in the laboratory (Elphick and Shine, unpubl. data). Hence, it is reasonable to extrapolate our laboratory results to the field: incubation at the 'hot' versus 'cold' extremes of natural nests will generate significant divergence in hatchling phenotypes (in terms of both morphology and locomotor performance), and this divergence will persist through a significant proportion of the young lizards' lives.

Although the effects of incubation temperatures on hatchling morphology are clear-cut (Table 1), the underlying mechanisms are less clear. On a proximate level, the smaller size of the 'cold'-incubated hatchlings may be due to their tendency to leave more yolk behind in the egg at the time of hatching. We did not quantify this variable, but our impression is that larger quantities of yolk remained in the eggs of cold-incubated hatchlings than in eggs that hatched from the 'hot' incubator. Hence, cold-incubated hatchlings may have been smaller because they did not internalize as much yolk during development. Several studies have documented larger quantities of unused yolk left behind in the eggs of reptilian hatchlings from 'cold' incubation treatments (Burger, Zappalorti & Gochfeld, 1987; Werner, 1988), though other studies have found more unused yolk in eggs incubated at higher temperatures (Phillips *et al.*, 1990; Vleck, unpubl. data, cited in Deeming & Ferguson, 1991). The adaptive significance of this pattern is unclear, but may involve hatching 'early' (i.e. at an earlier developmental stage, with more unused yolk) if incubation temperatures are unfavourable (i.e. are too high for optimal embryogenesis, or are so low that hatching will otherwise be delayed beyond the optimum time under field conditions).

Incubation-induced changes to the locomotor performance of hatchlings are even more difficult to interpret. Longer and heavier *B. duperreyi* ran faster than their smaller siblings, regardless of the incubation treatment to which they had been assigned. This result is consistent with virtually all studies of reptilian locomotor performance to date. Generally, within and between species, running speed increases with body size (e.g. Huey, 1982; Huey & Hertz, 1982; Garland, 1984, 1985; Garland & Huey, 1987; Dunham, Miles & Reznick, 1988; Daniels & Heatwole, 1990; Huey *et al.*, 1990; Losos, 1990a,b,c; Sinervo, 1990). Nonetheless, the body-size differences generated by our incubation treatments cannot explain why our 'hot'-incubated lizards ran faster than our 'cold'-incubated lizards. Firstly, morphological differences due to incubation treatment were relatively short-lived (see above), and thus could

not explain the longterm superiority in locomotor performance of the 'hot'-incubated lizards. Secondly, our ANCOVA analyses showed that 'hot'-incubated lizards were faster runners than their 'cold'-incubated siblings at identical body sizes (masses, SVLs, tail lengths).

If the morphological differences we documented cannot explain the disparity in locomotor performance, what can? Presumably, there may be more subtle morphological changes (perhaps relating to the types of muscle fibres laid down during development—e.g. Withers, 1992), or physiological shifts involving factors such as energy supply to the muscles. For example, acclimation to low temperatures permanently depresses speed performance in some ectotherms (e.g. Gatten, Echternacht & Wilson, 1988; Londos & Brooks, 1988). The same phenomenon may occur during embryonic life.

Persistence of incubation-induced modifications

The magnitude of the complex morphological response to incubation treatment decreased gradually during ontogeny (Tables 1 and 2). Although 'hot'-incubated *Bassiana duperreyi* hatchlings were heavier than their 'cold'-incubated siblings, there were no significant differences in body size or shape between the two treatment groups by about 6 weeks post-hatching (although hot-incubated offspring always maintained a size advantage over the 'cold' lizards when measured at the same calendar date). In contrast, the other main effect of the incubation treatment—on the locomotor performance of offspring—showed no signs of decreasing in magnitude throughout our study (Figs 1 and 2). 'Hot'-incubated *B. duperreyi* were faster runners than their 'cold'-incubated siblings, under all of our test conditions (four ages, two distances, and four body temperatures). The superior locomotor performance of 'hot' lizards was first observed at 2 weeks of age, and persisted until the completion of our study (by which time the lizards were 5 months old). The only incubation-induced aspect of escape behaviour that decreased ontogenetically was the frequency of the 'raised-tail-wag' tactic, which was seen primarily in very young lizards.

The adaptive significance of these performance effects remains obscure. As for morphology, it may be that an embryo developing in a 'cool' nest benefits from hatching earlier (because of ecological factors such as the approaching winter) to such a degree that it is 'worth' compromising locomotor performance, on a permanent basis, in order to complete development more rapidly. Alternatively, there may be no such adaptive value; the observed effects may be simple, non-adaptive consequences of different developmental pathways induced by incubation temperatures.

Consistency of incubation-induced modifications

Burger (1990) suggested that the temperature at which a reptile embryo is incubated determines a thermal 'set point' for the lifetime of the animal. In other words, the temperature experienced during embryogenesis determines the thermal optima for subsequent behavioural and physiological responses of that individual. This hypothesis predicts that lizards incubated in our 'hot' treatment would run faster at body temperatures close to their mean incubation temperature (i.e. at 25 and 30°C), but be outperformed by lizards from the 'cold' incubator when tested

at lower body temperatures (i.e. at 15 and 20°C). Our data did not support this ‘set point’ hypothesis: ‘hot’-incubated lizards performed better not only at higher body temperatures, but also consistently outran ‘cold’-incubated lizards when tested at the lower body temperatures. This result reflects the very high repeatabilities for most performance traits, both with time and across different test conditions (Shine & Elphick, unpubl. data). Because of these high repeatabilities, ‘running speed’ was essentially a single trait, with individuals relatively consistent in their ranking regardless of their ages or the temperatures at which they were tested. Nonetheless, we did detect a statistically significant interaction between incubation regime and the effect of different test temperatures on running speeds, at least over 1 m. This result suggests that the incubation-induced phenotypic modifications differentially affected locomotor ability under different test conditions. The magnitude of the effect was small, however, compared to the difference between lizards from the two incubation treatments (Figs 1 and 2). Thus, it seems that ‘hot’ nests produce offspring that are phenotypically superior, at least in terms of locomotor ability over the first few months of life.

Ecological significance of incubation effects

Although we chose the variables studied (i.e. incubation period, body size and shape, locomotor abilities) on the basis of their potential consequences for offspring fitness, it is impossible to specify the ‘optimal’ size, shape, running speed, or developmental period for *Bassiana duperreyi*. It seems intuitively reasonable that a bigger hatchling is ‘better’, that hatching earlier is advantageous, and that high running speeds enhance survival rates. Studies on other reptile species provide evidence for all of these assertions (see references below), but it is important to recognize that few such studies have been conducted—certainly, far too few to establish any generalities on the links between hatchling phenotypes and subsequent lifetime reproductive success. Thus, we can only speculate on the link between incubation temperature and fitness in *B. duperreyi*.

For a montane species such as *B. duperreyi*, living close to the upper elevational limit for oviparous reptiles in Australia (Pengilley, 1972; Shine, 1983), incubation period is likely to influence hatchling fitness. Eggs incubating at low temperatures may not hatch before the onset of harsh winter conditions. Meteorological records from the Brindabella Ranges indicate that nest temperatures in some years are too low for the successful completion of incubation (Shine, 1983). Additionally, the longer that eggs remain in the nest, the longer they are vulnerable to the threat of predation or physical damage (Andrews, 1982; Snow, 1982). A further disadvantage of long incubation periods in cold climates might be that late-hatching offspring would face reduced feeding opportunities, and so have to overwinter with lower fat reserves (Shine, 1983; Van Damme *et al.*, 1992).

Cold-incubated hatchlings not only emerged later, but were smaller than the ‘hot’ hatchlings. As with incubation period, there is a link between offspring size and fitness in some reptiles (Froese & Burghardt, 1974; Fox, 1975, 1978; Swingland & Coe, 1979; Ferguson, Brown & DeMarco, 1982; Ferguson & Fox, 1984; Janzen, 1993a). Ferguson & Fox (1984) attributed the survival advantage of larger juvenile side-blotched lizards (*Uta stansburiana*) to a competitive advantage in food acquisition, and not being as vulnerable to predation as their smaller conspecifics. Survival

advantages of large hatchling size have generally been attributed to these kinds of social and ecological factors (Froese & Burghardt, 1974; Fox, 1978; Janzen, 1993a). Surprisingly, we found a similar enhancement of survival rates for *Bassiana duperreyi* that were larger at hatching, independent of incubation conditions. Since our lizards were all maintained individually, the differential size-related mortality cannot be attributed to social factors or vulnerability to predation. This result could be interpreted as support for the 'bigger is better' hypothesis (i.e. that natural selection favours larger hatchlings), but it is difficult to interpret this pattern without further knowledge on the causes of mortality in our laboratory-raised hatchlings.

Could these high mortality rates of our captive lizards confound interpretation of the results? For example, could nonrandom mortality (e.g. of larger or faster lizards) within each group, combined with mortality differences between treatment groups, have generated the patterns that we observed? No, they did not. The direction and magnitude of incubation-induced differences remained relatively constant through time, in most traits that we measured (Tables 1, 2 and 3; Figs 1 and 2). Thus, differential mortality cannot explain the major results of our study.

Do lizards that run faster have a survival advantage that translates into differential fitness? Whilst it seems intuitively reasonable that 'faster is better', to date only a few studies have formally qualified the effect of 'superior' locomotor performance on survivorship in the field (Taylor & McPhail, 1985; Snell *et al.*, 1988; Bennett, 1990; Jayne & Bennett, 1990). If being faster is 'better', then it follows that being a faster runner over a range of body temperatures would also be 'better'. Individual *B. duperreyi* tend to maintain their relative ranking in performance capacity, even though absolute levels of performance are greatly affected by body temperature (as also shown in other reptile species—Bennett, 1980; Huey & Hertz, 1984; Else & Bennett, 1987; Huey & Dunham, 1987; van Berkum *et al.*, 1989). These results suggest that there are no necessary trade-offs in performance at higher or lower temperatures, and that *Bassiana duperreyi* are not thermal specialists, but are in fact 'jack-of-all-temperatures' (i.e. the 'hot'-incubated lizards out-perform 'cold'-incubated siblings at all test temperatures: see Huey & Hertz, 1984; Bennett, 1990). Again, however, we cannot be certain of any link between locomotor performance and lifetime reproductive success.

In summary, our data show that the thermal regimes characteristic of natural nests can induce major modifications to the phenotypes of hatchling lizards. These modifications involve morphology, locomotor ability, and anti-predator 'tactics'. At least under laboratory conditions, hatchling survival is also affected by the thermal regimes experienced prior to hatching. Previous work has shown that incubation temperatures may also affect other traits (such as activity levels and thermal preference) in *B. duperreyi* (Shine, 1995). Especially in the case of locomotor performance, these incubation-induced effects may be relatively long-lasting. In the absence of field data on lifetime reproductive success, we can only speculate on the ecological and evolutionary significance of these incubation effects. Nonetheless, available data are sufficient to suggest that much of the biologically important variation within a cohort of hatchling lizards is directly engendered by the physical conditions in natural nest sites, rather than reflecting underlying genetic variation. The strong contribution of phenotypic plasticity to this overall variation may well have important evolutionary implications, by decoupling natural selection from longterm evolution.

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