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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Potential targets for selection during the evolution of viviparity in cold-climate reptiles

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Abstract Viviparity (live-bearing) has evolved from oviparity (egg-laying) in more than 100 lineages of squamate reptiles (lizards and snakes). This transition generally has occurred in cool climates, where thermal differentials between eggs in the (cool) nest versus the (warm) maternal oviduct influence embryonic development, in ways that may enhance offspring fitness. To identify specific traits potentially under selection, we incubated eggs of a montane scincid lizard at conditions simulating natural nests, maternal body temperatures, and an intermediate stage (2-week uterine retention of eggs prior to laying). Incubation at maternal temperatures throughout incubation affected the hatchling lizard's activity level and boldness, as well as its developmental rate, morphology, and locomotor ability. A treatment that mimicked the initial stages of the transition toward viviparity had a major effect on some hatchling traits (locomotor speeds), a minor effect on others (tail length, total incubation period) and no effect on yet others (offspring behaviors). More generally, different aspects of the phenotype are sensitive to incubation conditions at different stages of development; thus, the evolution of reptilian viviparity may have been driven by a succession of advantages that accrued at different stages of embryogenesis.

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Introduction

The interplay between phenotypic plasticity and adaptive change has attracted scientific attention for well over a century (Scheiner 2014). Often, a novel environmental challenge will induce a phenotypically plastic response; if the resultant phenotypic variation translates into differential fitness, selection may then mold the trait by modifying the norm of reaction, or by inducing behaviors that generate the reaction norm that in turn produces optimal phenotypes (e.g., Ghalambor et al. 2007). The evolution of reptilian viviparity in cool climates offers a classic example of this process. Higher incubation temperatures for eggs retained in utero rather than laid in an external nest influence developmental trajectories and enhance offspring fitness (Shine 1995, 2002, 2004; Radder et al. 2008). In essence, viviparity evolves because a reproducing female reptile in a cool climate can manipulate the developmental environment of her progeny, inducing phenotypically plastic responses that increase offspring fitness (Shine 1995; Shine and Harlow 1996).

More than 100 phylogenetic lineages of squamate reptiles have undergone the transition from egg-laying to live-bearing, and comparative analyses suggest that in almost all cases, this transition has occurred as taxa invade cool climates (Shine 1983; Blackburn 1985; Li et al. 2009; Pincheira-Donoso et al. 2013); a contrary conclusion by Pyron and Burbrink (2014) has been refuted by further analysis (King and Lee 2015; Griffith et al. 2015). Experimental incubation of eggs (both in the laboratory and the field) has shown that retention of developing embryos at maternal rather than nest temperature modifies hatchling phenotypes (Shine 2002). Plausibly, such modifications can enhance offspring fitness and, thus, select for progressive increases in the duration of uterine retention of developing eggs (Shine 2002; Rodríguez-Díaz et al. 2010). The evidence for an adaptive significance to these shifts ranges from strong (egg survival: Shine 2002), to plausible (offspring running speeds, body shape e.g., Shine and Elphick 2001; Radder et al. 2008). Behavioral traits have attracted less attention than morphology or locomotor performance, but may also be sensitive to incubation conditions (activity level and diel timing, basking behavior, antipredator tactics, cognition: Shine and Harlow 1993; Shine 1995; Qualls and Shine 1996; Amiel and Shine 2012; but see Du et al. 2010).

Individual differences in behavior also may influence fitness (Boon et al. 2007; Sih et al. 2004; Carazo et al. 2014; Patrick and Weimerskirch 2015; Li et al. 2016). Such behaviors likely are widespread, although as yet documented in relatively few squamate reptiles (Bajer et al. 2015; McEvoy et al. 2015; Li et al. 2016); the sensitivity of such behavior to incubation temperature remains unstudied. Hence, we assayed the impact of incubation temperatures on hatchling behavior (as well as developmental rate, morphology, and locomotor performance) in a montane scincid lizard. Our larger aim was to assess a broad suite of phenotypic traits to ask if the thermal shift from a cold nest to a warm uterus influences each of these traits; if so, whether an intermediate stage between normal oviparity and viviparity (prolonged uterine retention of eggs) has equal impacts on each type of trait. If not, the selective advantage of increasing durations of uterine retention of eggs may shift through gestation; some benefits may accrue with only brief retention, whereas others require longer-term incubation at maternal temperatures.

Materials and methods

Collection and incubation of eggs

Three-lined skinks (*Bassiana duperreyi*) are medium-sized (up to 80 mm snout-vent length [=SVL]) oviparous terrestrial lizards widely distributed through cool-climate montane habitats in south-eastern Australia (Cogger 2014). Extensive previous studies on this species provide essential background data on critical issues such as thermal regimes in natural nests, and the body temperatures selected by gravid females (e.g., Shine and Harlow 1996). In high-elevation sites close to the upper elevational limit for oviparous reproduction by Australian lizards (Shine and Harlow 1996), *B. duperreyi* nest communally in the few open areas where sunlight penetration warms the soil (Shine et al. 2002; Radder and Shine 2007). Each female lays a single annual clutch of 3–9 eggs under logs or rocks in early summer (late November or early December). Oviposition is highly synchronous, which enables us to collect large numbers of eggs soon after females finish laying (Shine and Harlow 1996). On Dec 10, 2014, we collected *B. duperreyi* eggs from Coree Flats (1060 m asl, 35°16′56″S, 148°48′29″E) in the Brindabella Range 40 km west of Canberra, in the Australian Capital Territory.

Eggs were transported to the University of Sydney where they were weighed individually and then placed into 64-ml glass jars filled with moist vermiculite (water potential −200 kPa) and sealed with plastic cling wrap. Eggs from each communal nest were randomly divided among four incubators, with thermal regimes that varied on a daily cycle. Each incubation treatment was based on extensive field data (see Shine et al. 2003) and was replicated in two incubators. One treatment mimicked regimes measured in natural nests ("oviparity," 20 ± 5 °C), and another mimicked maternal body temperatures ("viviparity," 27 ± 5 °C). We also created a third treatment to simulate an intermediate stage between oviparity and viviparity (eggs retained in utero, then laid in a nest): eggs in this "uterine retention" treatment spent 2 weeks in a "viviparity" incubator (at 27 ± 5 °C), then the remainder of incubation in an "oviparity" incubator (at 20 ± 5 °C). We monitored temperatures inside the incubators (Clayson IM550R 10-step incubators; Brisbane, Queensland, Australia) using iButton thermochrons (Maxim Integrated Products, Dallas, TX) recording at 15-min intervals.

Morphology, husbandry, and locomotor performance of hatchlings

Upon hatching, lizards were weighed using a Sartorius top-loading balance (Gottingen, Germany), accurate to ±0.001 g. Hatchlings were then transferred to a 64-ml glass jar and placed in ice for 15 min to cool down to a temperature where they remained immobile for several minutes. We were then able to measure hatchling SVL and total length with a transparent plastic ruler (±0.5 mm). Hatchling sex was identified by applying pressure to the tailbase with forceps to record the presence/absence of hemipenes, under a binocular microscope (Shine and Harlow 1996).

Hatchlings were housed individually in plastic cages (200 × 150 × 70 mm) containing paper towel as substrate, a water dish, and a 10 cm length of plastic pipe for shelter. Air temperature was kept at 20 ± 0.5 °C, but heating strips beneath each box allowed hatchlings to select higher temperatures (to 35 °C) for up to 10 h per day. The light cycle was set from 0600 to 2000 hours, similar to field conditions. Hatchlings were fed vitamin-dusted crickets (*Acheta domesticus*) every second day, and had constant access to water.

At seven days of age, hatchling locomotor performance was measured on a racetrack (1 m long and 4 cm wide) at 24 °C (close to the usual activity body temperature for this species: Shine 1983). After 30 min acclimation to the test temperature, each lizard was placed at one end of the track, and encouraged to run the 1-m distance using a soft-bristled paintbrush. Individual lizards were run three times with 15-min rest between successive trials (and kept at the same temperature between successive trials). Running speed (m s^{-1}) was recorded using an infrared timing device, fitted with photocells at 25-cm intervals along the track. Mean burst speed (fastest 25-cm segment) and mean sprint speed (over 1 m) were calculated for each hatchling.

Trials of hatchling behavior

At 10 days of age, 20 male and 20 female hatchlings were randomly selected from each thermal treatment (one per clutch, to avoid pseudoreplication). We conducted two kinds of tests on each lizard (activity levels and boldness trials).

Activity levels

We measured activity levels in an open arena, under three different conditions. Two of the trials (the first and the last) were identical, and both were designed to measure exploration (empty arena); a second type of trial was designed to assess neophobia (novel stimulus within the arena), and a third was designed to measure sociality (conspecific already present within the arena).

For each trial, a lizard was placed in a standardized location (4 cm from one wall) in an open arena (328 × 270 × 120 mm) that also held a transparent plastic container (250 ml) positioned 8 cm from one of the other walls. For boldness trials, the plastic container (250 ml) was empty. For neophobia trials, the container covered a plastic fishing lure with waving arms (moved by a small motor). For sociality trials, the container sheltered a conspecific lizard.

Boldness trials

We also ran trials in which we recorded the duration of time before a lizard emerged from a shelter into the open arena (328 × 270 × 120 mm) to measure boldness. For boldness trials, the lizard was first placed within an inverted cup with a single exit hole (rather than simply being put down in the open).

Trial order and video analysis

Each lizard was tested in the same order (exploration trial 1, neophobia trial, boldness trial, sociality trial, exploration trial 2) over 5 consecutive days, and at the same time of day. Prior to trials, all hatchlings were acclimated to the temperature (26 °C) for at least 30 min. Video cameras recorded each trial for 15 min, after which time the lizards were returned to their home enclosure. The videos were analyzed with automated image-based tracking software (Ethovision XT 10.0, Noldus Information Technology, the Netherlands).

After completion of the experiment, all lizards were returned to the field and released beside their nest of origin.

Statistical analyses

Behavioral trials

Data on behavioral traits were non-normally distributed. To achieve normality and reduce the numbers of correlated variables, we conducted principal components analysis on the three activity measures (distance moved, mean velocity, and cumulative duration of movement, which were $\log_{10}(x + 1)$ transformed) from all four of the activity trials. The first principal components axis (PC1) explained 85.1 % of the total variation in the dataset and was positively correlated with all three of the activity-related traits we scored (in all of these cases, $r > 0.70$), suggesting that PC1 provides a robust index of a lizard's overall activity level. Also, PC1 was normally distributed. We thus used PC1 as an index of activity level, and investigated thermal treatments differences in activity levels with ANCOVA using PC1 as the dependent variable, thermal treatment and trial type as the factors. Animal ID was included as a random variable to control for pseudoreplication. Data on boldness (time to emerge from a shelter) were examined using ANOVA, with thermal treatment as the factor and time to emerge as the dependent variable. Where significant, analyses were followed by post hoc Tukey's HSD tests to locate meaningful differences. Then we used Pearson product-moment correlation coefficients to assess correlations among each individual's score on different trials, and also to compare an individual's behavior in "time to emergence trials" to its performance in the other three kinds of trials (neophobia, sociality, and boldness). The levels of statistical significance of such tests are questionable because multiple testing can lead to artifactually "significant" results; authorities disagree on whether or not such p values should be "corrected" (Schwager 1984; Nakagawa 2004). However, the r and p values provide useful information about patterns of covariation among traits.

Incubation effects

Initial analyses included incubator nested within treatment as an additional factor, but no significant incubator effects were detected, and there were no significant differences between sexes among any behavior traits either (all $p > 0.18$). Thus, we report the simpler (one-factor) analyses below. We examined differences among treatments (in incubation period, lizard morphology, locomotion, and behavior scores) using one-factor ANOVA (for continuous variables) followed by post hoc Tukey's HSD tests to locate significant differences. Data analyses were performed with JMP, version 11.0 (SAS Institute, Cary, NC). All values in figures are presented as mean ± SE, and the significance level was set at $\alpha = 0.05$.

Results

Activity level

Individual lizards were highly consistent in behavior across all trials. Pearson product-moment correlation coefficients between different descriptors of lizard behavior in different trial types were almost all statistically significant (Table 1; Table 2). This consistency suggests that *B. duperreyi* display behavioral syndromes: an individual that was highly active in one type of trial was also active in others, and emerged sooner from its shelter (Table 3). Hatchlings from the "viviparity" (V) treatment were more active than their siblings from the other two thermal treatments (Table 3; Fig. 3). We found significant effects of treatment and trial category on all three variables (distance moved, mean velocity, cumulative duration of movement). Using PC1 as an index of activity level, ANOVA revealed strong differences between hatchlings from the "viviparity" treatment versus those from either "uterine retention" or "oviparity" treatments (mean ± SE = 0.77 ± 0.12, −0.24 ± 0.12, −0.53 ± 0.12, respectively; $F_{2,472} = 33.34$, $p < 0.0001$). Post-hoc Tukey's HSD tests revealed significant differences between all three treatments (viviparity, uterine retention, and oviparity).

Incubation effects

Incubation treatment affected some but not all of the hatchling traits we measured, and the nature of differences elicited by thermal treatments varied among traits.

Incubation period

The duration of incubation was about twice as long for the "oviparity" (O) as the "viviparity" (V) treatment, with the "uterine retention" (UR) treatment incubation period midway between these two extremes (ANOVA, $F_{2,118} = 919.03$, $P < 0.001$, post-hoc O > UR > V; Fig. 1a).

Morphology and locomotor performance

The offspring sex ratio averaged close to 50:50 and was not affected by incubation treatment (logistic regression $\chi^2 = 0.16$, $df = 2$, $P = 0.93$; Fig. 1b). Similarly, offspring body length (SVL) was unaffected by treatment ($F_{2,118} = 2.17$, $P = 0.12$; Fig. 1c). Tail length was shortest in hatchlings from the oviparous treatment and longest in those from the viviparity treatment ($F_{2,118} = 4.95$, $P < 0.01$, post-hoc V > O; Fig. 1d).

Offspring running speed was lower for hatchlings from the "oviparity" treatment than either of the other two groups over both test distances (25 cm, $F_{2,118} = 62.75$, $P < 0.001$, post hoc V = UR > O; Fig. 2a; 1 m, $F_{2,118} = 105.31$, $P < 0.001$, post-hoc V = UR > O; Fig. 2b).

Boldness test

The duration of time before a lizard emerged from a shelter into the open arena showed the same pattern as activity level (above); hatchlings from the "viviparity" treatment emerged sooner than did hatchlings from the other two treatments (Table 3; Fig. 4). This result suggests that hatchlings from the "viviparity" (V) treatment were bolder than those from the "oviparity" (O) and the "uterine retention" (UR) treatments.

Overall treatment effects

When we included all of these dependent variables (i.e., incubation period, morphology, locomotor performance, activity level, time to emergence) in a single MANOVA, the analysis detected strong differences in the way that treatment affected different variables (treatment*variable Wilk's Lambda, $F_{22,186} = 35.28$, $P < 0.001$). The most interesting comparison involves traits that differed between the "oviparity" and "viviparity" treatments, and hence might be involved in selection for progressive increases in the duration of uterine retention of developing eggs. Even brief retention at high temperatures was enough to generate a fast-running hatchling, and prolonged retention conferred little or no extra benefit in running speed (Fig. 2). In contrast, a 2-week retention at higher temperatures had little or no impact on behavioral syndromes (activity level and emergence time); these traits were affected only by a more prolonged retention (Figs. 3, 4). Lastly, traits such as incubation period and tail length were affected to an intermediate degree; "uterine retention" generated progeny with traits halfway between those of the "oviparity" and "viviparity" treatments (Fig. 1a, d).

Table 1 Pearson product-moment correlation coefficients to assess the consistency of individual variation in behavior as measured in four types of trials for three-lined skinks (*Bassiana duperreyi*)

Behavior trials	E-DM	E-MV	E-CMD (%)	N-DM	N-MV	N-CMD (%)	S-DM	S-MV	S-CMD (%)	B-THO	B-TBLO	B-TFBO
E-DM												
E-MV	$r = 0.77$ $P < 0.001$											
E-CMD (%)	$r = 0.92$ $P < 0.001$	$r = 0.80$ $P < 0.001$										
N-DM	$r = 0.31$ $P < 0.001$	$r = 0.39$ $P < 0.001$	$r = 0.36$ $P < 0.001$									
N-MV	$r = 0.17$ $\boldsymbol{P = 0.064}$	$r = 0.19$ $P < 0.042$	$r = 0.16$ $\boldsymbol{P < 0.09}$	$r = 0.37$ $P < 0.001$								
N-CMD (%)	$r = 0.33$ $P < 0.001$	$r = 0.35$ $P < 0.001$	$r = 0.35$ $P < 0.001$	$r = 0.87$ $P < 0.001$	$r = 0.26$ $P = 0.005$							
S-DM	$r = 0.44$ $P < 0.001$	$r = 0.48$ $P < 0.001$	$r = 0.48$ $P < 0.001$	$r = 0.51$ $P < 0.001$	$r = 0.25$ $P = 0.005$	$r = 0.42$ $P < 0.001$						
S-MV	$r = 0.51$ $P < 0.001$	$r = 0.57$ $P < 0.001$	$r = 0.56$ $P < 0.001$	$r = 0.49$ $P < 0.001$	$r = 0.32$ $P < 0.001$	$r = 0.47$ $P < 0.001$	$r = 0.79$ $P < 0.001$					
S-CMD (%)	$r = 0.39$ $P < 0.001$	$r = 0.43$ $P < 0.001$	$r = 0.40$ $P < 0.001$	$r = 0.42$ $P < 0.001$	$r = 0.31$ $P < 0.001$	$r = 0.37$ $P < 0.001$	$r = 0.91$ $P < 0.001$	$r = 0.78$ $P < 0.001$				
B-THO	$r = -0.21$ $P < 0.03$	$r = -0.26$ $P < 0.005$	$r = -0.21$ $P = 0.023$	$r = -0.26$ $P < 0.005$	$r = -0.18$ $P = 0.049$	$r = -0.25$ $P < 0.007$	$r = -0.24$ $P = 0.008$	$r = -0.36$ $P < 0.002$	$r = -0.27$ $P = 0.003$			
B-TBLO	$r = -0.20$ $P = 0.033$	$r = -0.26$ $P < 0.004$	$r = -0.22$ $P = 0.018$	$r = -0.27$ $P = 0.003$	$r = -0.21$ $P = 0.025$	$r = -0.26$ $P = 0.005$	$r = -0.24$ $P < 0.010$	$r = -0.36$ $P < 0.001$	$r = -0.25$ $P < 0.006$	$r = 0.90$ $P < 0.001$		
B-TFBO	$r = -0.21$ $P = 0.02$	$r = -0.26$ $P = 0.004$	$r = -0.23$ $P < 0.012$	$r = -0.27$ $P < 0.004$	$r = -0.18$ $\boldsymbol{P = 0.056}$	$r = -0.25$ $P < 0.007$	$r = -0.22$ $P = 0.015$	$r = -0.36$ $P < 0.001$	$r = -0.22$ $P = 0.016$	$r = 0.86$ $P < 0.001$	$r = 0.96$ $P < 0.001$	

Values in bolditalic show results that are not statistically significant ($P > 0.05$)

E exploration trial, *N* neophobia trial, *S* sociality trial, *B* boldness trial, *DM* distance moved, *MV* mean velocity, *CMD* cumulative duration of movement (%), *THO* time for head out, *TBLO* time for back leg out, *TFBO* time for full body out

Table 2 Pearson product-moment correlation coefficients between traits assessed in "boldness" trials compared to the mean values of behavioral traits in the other four types of trials conducted on three-lined skinks (*Bassiana duperreyi*)

Behavior traits	Time for head out	Time for back leg out	Time for full body out
Total distance moved	$r = -0.29$ $P < 0.002$	$r = -0.29$ $P < 0.002$	$r = -0.29$ $P < 0.002$
Mean velocity	$r = -0.33$ $P < 0.001$	$r = -0.35$ $P < 0.001$	$r = -0.33$ $P < 0.001$
Cumulative duration of movement (%)	$r = -0.31$ $P < 0.001$	$r = -0.31$ $P < 0.001$	$r = -0.30$ $P < 0.001$

Table 3 The effects of incubation treatment on the boldness of hatchling lizards, as quantified by the time taken to emerge from a shelter

Behavior scores	$F_{2,116}$	P	Post hoc comparison
Time for head out	5.17	0.007	O = UR > V
Time for back leg out	6.72	<0.002	O = UR > V
Time for full body out	6.11	0.003	O = UR > V

The table reports values from one-way ANOVA

V viviparity treatment, *UR* uterine retention treatment, *O* oviparity treatment

Discussion

Our results show that hatchling skinks exhibit consistent individual variation in behavioral traits both when re-tested using the same protocol, and when exposed to different protocols. Thus, they show "behavioral syndromes," as do most other kinds of animals that have been tested in this way (Mazué et al. 2015). The consistency was evident in all traits that we measured. Our data thus adds hatchling behaviors (activity level and boldness) to the list of hatchling traits that can be modified by thermal conditions in the nest.

Since the thermal conditions we applied to eggs closely mimic those recorded in the field (e.g., Shine et al. 2003), any increase in the duration of uterine retention of eggs—whether induced by plasticity (Telemeco et al. 2010) or adaptation (Shine 2002)—is likely to have profound impacts on the phenotypic traits of hatchling lizards. In the case of *B. duperreyi*, experimental manipulation of incubation thermal regimes suggests that a maternally retained egg is likely to give rise to a faster running, smarter, more active animal than would have emerged from a nest in the field (Shine 2002; Shine and Olsson 2003; Amiel and Shine 2012; current study).

Importantly, however, the impact of high-temperature incubation appears to differ among traits. Retention for the

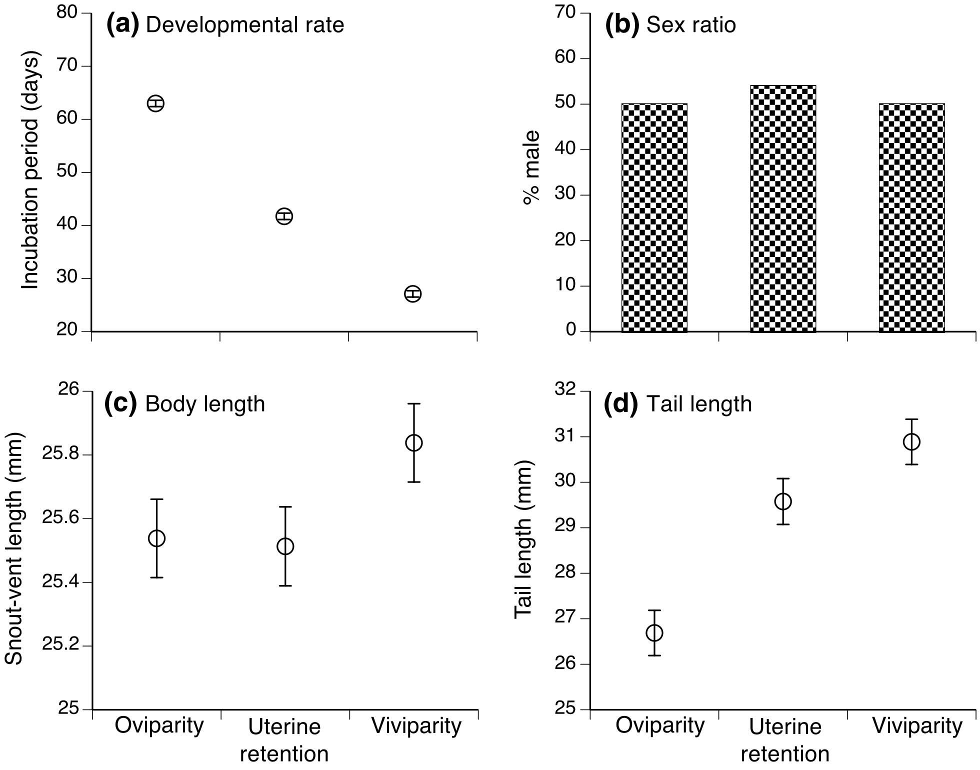

Fig. 1 The effect of different thermal regimes during incubation on the development of skinks, *Bassiana duperreyi*. Eggs collected shortly after they were laid in the field were kept at conditions mimicking natural nests ("oviparity", cool), uterine retention of eggs for 2 weeks ("uterine retention", warm then cool), and uterine retention through development ("viviparity", warm). The panels show **a** total incubation period, **b** sex ratio, **c** snout-vent length, and **d** tail length of the hatchlings. All graphs show mean values and associated standard errors

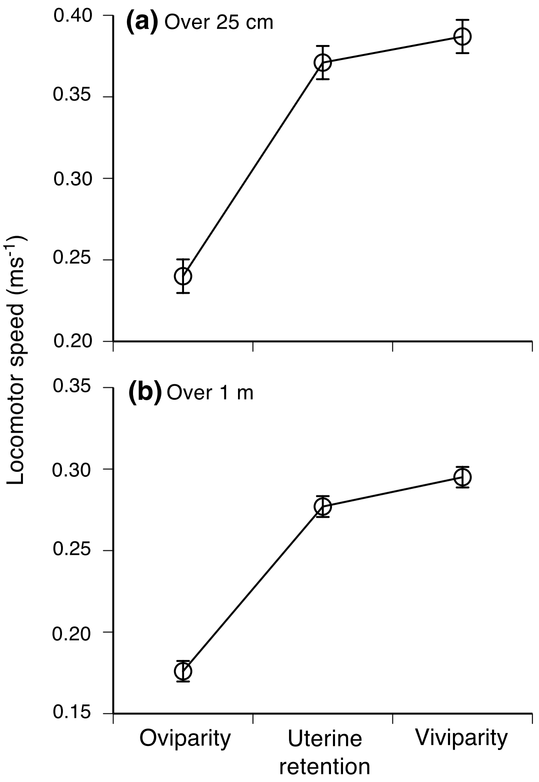

Fig. 2 The effect of different thermal regimes during incubation of skinks, *Bassiana duperreyi*, on running speeds of hatchlings over **a** 25 cm and **b** over 1 m. Eggs collected shortly after they were laid in the field were kept at conditions mimicking natural nests ("oviparity", cool), uterine retention of eggs for 2 weeks ("uterine retention", warm then cool), and uterine retention through development ("viviparity", warm). All graphs show mean values and associated standard errors

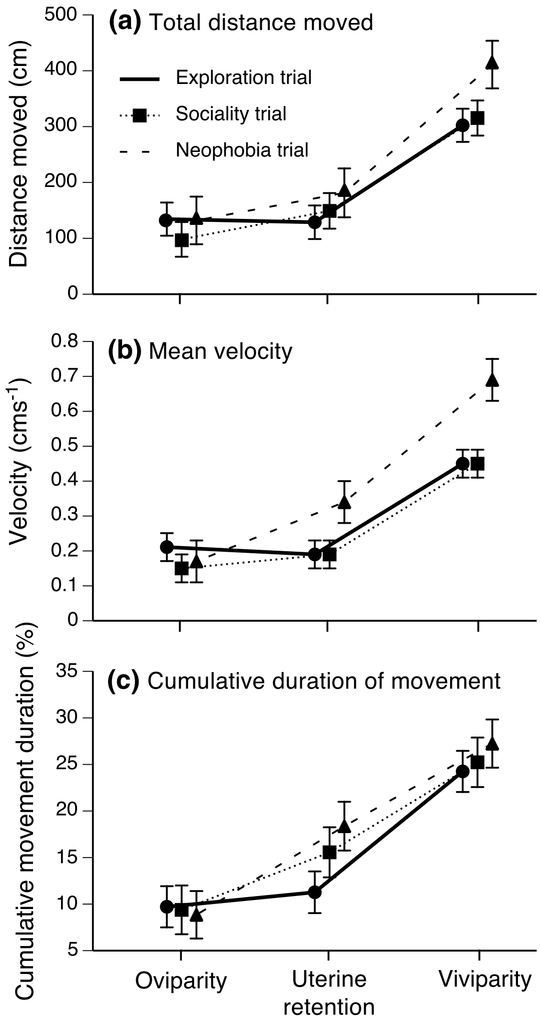

Fig. 3 The effect of different thermal regimes during incubation of skinks, *Bassiana duperreyi*, on activity levels of hatchlings in standardized trials. Eggs collected shortly after they were laid in the field were kept at conditions mimicking natural nests ("oviparity", cool), uterine retention of eggs for 2 weeks ("uterine retention", warm then cool), and uterine retention through development ("viviparity", warm). Each hatchling was tested in four trials, in a consistent order, to assess exploration, then neophobia, then sociality, then exploration again. We calculated **a** total distances moved, **b** mean velocity, and **c** cumulative duration of movement (%) during each 15-min behavior trial. The graph shows mean values and associated standard errors

entire incubation period changed many phenotypic traits, but the effect of a briefer (2-week) retention at high temperatures was negligible on some traits (hatchling activity level and boldness), minor on others (tail length and development time), and major on yet others (running speed). This result accords well with basic principles of developmental biology. Since embryogenesis involves the progressive elaboration of organ systems, environmental modifications at different embryonic stages are likely to influence different traits (Johnston 2006). For example, environmental disruptions early in embryogenesis have more impact than do those occurring later (Gilbert 2000; Shine and Elphick 2001; Shine 2004; Löwenborg et al. 2011). Many traits are sensitive to incubation conditions only during some specific "window"; for example, in reptiles with temperature-dependent sex determination, the middle third of incubation is often the most critical in determining offspring sex (Deeming and Ferguson 1988; Deeming 2004; Neaves et al. 2006; Shine et al. 2007; Warner and Shine 2011). Similarly, the effect of hot weather on the morphology of neonatal vipers (*Vipera aspis*) depended on the time of year at which those conditions occurred (Lourdais et al. 2004).

Many studies have shown links between behaviors and fitness-linked traits, but the nature of those links depends upon local habitats. For example, more active individuals may be able to gather information about their surroundings more rapidly (Careau et al. 2009; Bajer et al. 2015), but may encounter higher risks if predators are abundant (Biro et al. 2004). In the present study, hatchlings that developed under warm conditions ("viviparity" treatment) were more active and ran faster than did their siblings from cooler

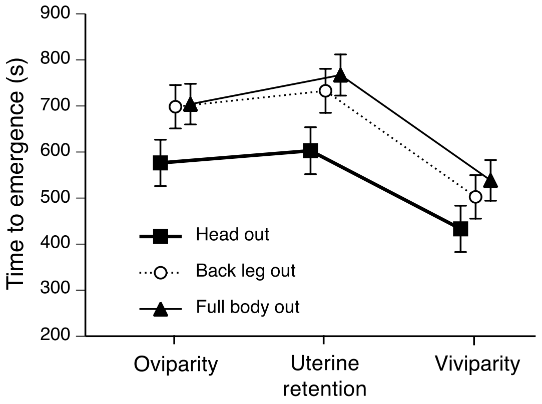

Fig. 4 The effect of different thermal regimes during incubation of skinks, *Bassiana duperreyi*, on the time taken for hatchlings to emerge from a shelter in standardized trials. Eggs collected shortly after they were laid in the field were kept at conditions mimicking natural nests ("oviparity", cool), uterine retention of eggs for 2 weeks ("uterine retention", warm then cool), and uterine retention through development ("viviparity", warm). Each hatchling was video-recorded to quantify the time taken for it to protrude its head, then body, then tail, from a shelter. The graph shows mean values and associated standard errors

incubation regimes. If selection favors faster, more active individuals (as may often be true: Elphick and Shine 1998; Careau et al. 2009; Bajer et al. 2015; Li et al. 2016) warmer incubation might thereby enhance offspring fitness.

In the case of *B. duperreyi*, traits that affect locomotor speed were sensitive to temperatures in early incubation, whereas those which affect personality (presumably, mediated by changes in brain structure and/or function) were sensitive at a later stage. Consistent with that result, an earlier study on *B. duperreyi* reported that hatchling antipredator tactics and basking behavior were affected by incubation temperature during late but not early development (Shine 1995). However, other behavioral traits show complex and sometimes conflicting results (Shine 1995; Qualls and Shine 1996). Based on the present study, the evolution of reptilian viviparity (via progressive increases in the duration of uterine retention of eggs) in a species like *B. duperreyi* may have involved a shifting kaleidoscope of selective forces. Enhanced locomotor performance may have driven the initial stages of uterine retention, but have been irrelevant to later increments in the duration of retention. Instead, those later phases may have been driven by effects of temperature on behavioral traits of the progeny. Throughout the entire progression from oviparity to viviparity, other advantages of high-temperature incubation—such as the acceleration of developmental rate, and shifts in body shape—may have played a more consistent role.

The speculations above assume a selective advantage to traits such as earlier hatching, faster running speed, and higher activity levels. We have no data to support that assumption. However, earlier hatching plausibly enhances offspring viability in this cold-climate species (Elphick and Shine 1998; Qualls and Andrews 1999), and faster locomotor speed is associated with higher survival rates in other lizards (Warner and Andrews 2002). Additionally, traits that we have treated as separate may interact—for example—within our dataset, lizards with longer tails also tended to be faster runners (over a distance of 1 m, $r^2 = 0.04$, $P < 0.03$). In practice, all of these components of the phenotype need to work with each other in an integrated fashion. For example, effective antipredator behavior involves early detection of the predator, an appropriate response tactic, locomotor speed, and so forth. Also, superficially, disparate traits may form parts of a functionally linked syndrome: for example, a longer tail may facilitate effective autotomy and thus protect the individual from attacks by predators (Medel et al. 1988). In some cases, these suites of traits may be induced by the same environmental factors during early ontogeny (Bestion et al. 2014). Although components of the hatchling phenotype may differ in the timing of their sensitivity to incubation conditions, the eventual viability (fitness) of the hatchling cannot be broken down into the effects of discrete traits.

The large number of independent phylogenetic transitions from oviparity to viviparity within lizards and snakes and the ready availability of eggs from many taxa provide a powerful model system with which to explore the nature of phenotypically plastic responses induced by evolutionary transitions in reproductive mode. The specific phenotypic traits affected by incubation conditions at different phases of incubation, and the fitness consequences of such variations, doubtless will differ considerably among taxa. For example, thermal differentials between maternal and nest temperatures may be lower in many tropical and thermoconforming taxa than in the heliothermic montane skinks that we studied. In some systems, thermal variance rather than mean temperatures may be important. Regardless of such diversity in responses, however, the broad result that incubation temperatures have profound and multifaceted impacts on the phenotype of a hatchling lizard likely is very widespread. The ease with which investigators can manipulate hatchling phenotypes provides exciting opportunities to explore developmental plasticity, and to conduct experimental rather than correlational studies on the fitness consequences of variation in animal behaviors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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