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ARE REPRODUCTIVE LIFE HISTORY TRAITS OF AUSTRALIAN

THREE-LINED SKINKS FIXED OR PHENOTYPICALLY PLASTIC?

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Are Reproductive Life History Traits of Australian Three-

lined Skinks Fixed or Phenotypically Plastic?

A THESIS

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ABSTRACT

Reptile viviparity is strongly associated with cool climates in interspecific comparisons, but intraspecific comparisons provide an even stronger opportunity to identify causal pathways and distinguish facultative (plastic) effects from canalised adaptive responses. I documented reproductive traits in the Australian three-lined skink (*Bassiana duperreyi*), an oviparous scincid lizard from alpine south-eastern Australia, and manipulated thermal regimes of captive gravid females and of their eggs to identify proximate causes of life-history variation. Embryonic developmental stages at oviposition were higher in eggs laid by females from high-elevation (1615-1670 m asl) populations than in eggs produced by females from lower elevation (1240 m asl). Furthermore, in the laboratory, experimentally-imposed low maternal body temperatures delayed oviposition and resulted in more advanced embryonic development at oviposition. Warm conditions both *in utero* and in the nest increased hatching success and offspring body size. In total, my results suggest much of the elevation-related variation in life-history traits among populations of *B. duperreyi* reflects plastic responses to different thermal environments, rather than canalized genetic differences.

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INTRODUCTION

The growing reality of climate change (IPCC 2007) is focusing scientific attention onto the question of how variation in ambient thermal conditions affects organisms (Visser 2008). An immense literature documents the importance of this linkage (e.g., Ji and Brana 1999; Deeming 2004; Shine 2005). For example, compared to their tropical relatives, cold-climate animals differ consistently in body sizes and shapes, physiological attributes, demographic traits and reproductive modes (Tinkle and Gibbons 1977; Van Damme et al. 1989; Grant and Dunham 1990; Ramirez-Bautista et al. 2002; Smith et al. 2003; Sears 2005; Calderon-Espinosa et al. 2006). Such variation may have fixed genetic causes (Reznick and Bryga 1996; Seigel and Ford 2001; Baker and Foster 2002), or reflect phenotypic plasticity (Shine 2003; Weitere et al. 2004; Du et al. 2005). Plastic responses to thermal conditions may be expressed during either ontogenetic development (i.e., temperature-dependent sex determination, TSD: Bull 1980), or as a result of exposure to specific thermal challenges (Sears and Angilletta 2003; Telemeco et al. 2009). Thus, answering the superficially simple question "how does climate affect organisms?" requires complex answers that differ among species and phenotypic traits.

Some model systems facilitate analysis of the linkage between climate and organismal traits. One such model is reproductive mode in squamate reptiles (lizards and snakes).

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Although most squamate species are oviparous (egg-laying),
the alternative reproductive mode of viviparity (live-
bearing) has evolved independently in approximately 100
lineages (Blackburn 1982, 1985; Shine 1985). The present-
day distributions of viviparous squamates are strongly
(albeit, not universally) associated with cold climates
(Tinkle and Gibbons 1977; Shine 1985; Calderon-Espinosa et
al. 2006), a pattern that is arguably the strongest single
correlation ever documented between a vertebrate
reproductive trait and an abiotic factor (Greer 1989; Shine
2005). In virtually all squamate taxa where evidence is
available to reconstruct the transition in reproductive
mode, colonization of regions with cold climates has been
implicated as a causal force in the evolutionary shift from
oviparity to viviparity (Shine 1985; Andrews et al. 1997;
Shine 2002). The selective pressures responsible for such
shifts have prompted considerable speculation (Mell 1929;
Sergeev 1940; Packard et al. 1977; Shine and Bull 1979;
Shine 1995; Andrews 2000), but the emerging consensus
involves advantages resulting from prolongation of maternal
control over embryonic developmental conditions (the
"maternal manipulation hypothesis": Shine 1995; Ji et al.
2002; Webb et al. 2006). In keeping with that hypothesis,
experimental exposure of eggs to thermal conditions
simulating putative intermediate stages between oviparity
and viviparity suggests that prolonged uterine retention of

eggs prior to laying enhances offspring fitness at high elevations but not at lower elevations (Shine 2002).

Although there is broad acceptance of the hypothesis that invasion of colder sites imposes selection for prolongation of uterine retention of eggs in squamate reptiles, there is surprisingly little intraspecific evidence to support this hypothesis. For example, under this hypothesis, elevational clines in the duration of uterine retention of eggs are expected within wide-ranging species; but in fact, few such cases have been documented (*Anolis cybotes*- Huey 1977; *Sceloporus scalaris*- Mathies and Andrews 1995; *Saiphos equalis*- Smith and Shine 1997). We might also expect facultative retention of uterine eggs under unusually cold conditions, similar to the precipitation-induced plasticity seen in species that await rainfall before oviposition (Andrews and Mathies 2000; Warner and Andrews 2003) but again, data are lacking. Most studies have reported or assumed that the degree of embryonic development at oviposition is a fixed trait within populations, rather than responsive to local thermal conditions (Shine 1985; Blackburn 2006). We are thus left with a paradox: well-supported theory and interspecific comparisons suggest that prolonged uterine retention of eggs enhances fitness under colder conditions, but there is little empirical evidence that cooler conditions prolong uterine retention of eggs at the intraspecific level either as a facultative response, or a canalised adaptation.

To explore this paradox, I examined the effects of cold conditions (both environmental and experimentally induced) on reproductive mode and offspring fitness in a montane scincid lizard that occupies a wide elevational (and therefore thermal) range. Because of its broad range, I predicted that this species will display intraspecific variation in reproductive mode such that high elevation females retain embryos longer than do low elevation females. My results supported this prediction (see below). To clarify whether this variation in egg retention results from adaptive or facultative responses to local thermal conditions, I exposed gravid females from high and low elevation populations to warm and cold temperatures in the laboratory, and staged eggs at oviposition. I also explored the adaptive significance of embryo retention by incubating eggs at hot and cold incubation temperatures and measuring the resulting offspring survivorship and phenotype. If viviparity is adaptive in cold environments because it allows females to increase incubation temperatures of their eggs, warmer temperatures both before and after oviposition are predicted to result in fitter offspring.

Figure 1: Female *Bassiana duperreyi* with nest in the laboratory

MATERIALS AND METHODS

Study Species and Populations

The Australian three-lined skink, *Bassiana duperreyi*(Fig 1), is a medium-sized (to 80 mm snout-vent length, SVL)oviparous lizard abundant throughout alpine habitats in south eastern Australia (Cogger 2000). Females lay a single clutch of three to nine eggs early in summer, under rocks or logs in open areas exposed to high levels of solar radiation (Shine and Harlow 1996). Oviposition among females within a population is highly synchronous, and communal oviposition is frequent (Pengilley 1972; Radder and Shine 2007). As in most oviparous squamate species studied to date, embryos are retained *in utero* for approximately one-third of the total developmental period prior to oviposition (Pengilley 1972; Shine et al. 1997; Radder et al. 2008).

My study populations were located in the Brindabella Ranges 40 km west of Canberra in the Australian Capital Territory. The populations were as follows: Picadilly Circus (PC, 1240 m asl, 148°50' E, 35°21' S), Mount Ginini (GN, 1615 m asl, 148°46' E, 35°32 S), and Mount Gingera (GG, 1670 m asl, 148°47'E, 35°34' S). The population locations were arranged in a north to south-running line with PC being northernmost, GN ~ 24 km south, and GG a further ~ 6 km south. These sites spanned ~ 430 m in elevation with the two higher sites close to the upper-elevational limit for oviparous reproduction by Australian lizards (Radder et al. 2007).

Thermal Regimes in Natural Nests

To determine the thermal regimes in natural nests at my three sites, I acquired data collected previously by the Shine Laboratory at the University of Sydney. These data were collected over multiple years (PC: 1997-2002, 2005, 2006; GN: 1997, 2000-2001, 2006; GG: 2001, 2005-2006) using miniature thermal data-loggers (thermochron ibuttons, Dallas Semiconductor, Dallas, TX; diameter 15 mm, height 6 mm, mass 3.3 g). Fewer years at the upper-elevation sites were monitored because access was denied to these sites some years due to fire concerns in the area. In total, 123 nests were monitored ($\bar{x}$ nests per year ± 1.0 *SE*: PC = 14 ± 2.4, GN = 2.3 ± 0.3, GG = 5 ± 3.5). Each year, trips were made in

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early summer to locate nests containing recently oviposited eggs (< 1 wk from oviposition, based on subsequent incubation periods in the laboratory). Data-loggers set to record temperature every hour were placed within nests at their mid-depths (determined for each nest). After nine weeks, which is the approximate incubation duration for *B. duperreyi* (Shine et al. 1997), the data-loggers were recovered and their data were downloaded for analysis. Unless stated otherwise, I used the software program Statview 5 (SAS Institute, Cary, NC) to analyse all data in this study. I used ANOVA to examine the effect of site on mean nest temperature over the entire 9-wk period. Because data were available from PC for 8 y versus only 4 y at GN and 3 y at GG, I used ANOVA to examine inter-annual variation in nest temperature only at PC.

Experiments with Adult Lizards

I collected adult *B. duperreyi* from each site during seven trips into the Brindabella Ranges from 9 October through 2 December 2007 (early in the reproductive season). I collected both males and females because I often was unable to distinguish if females had already ovulated (Total N = 44; PC female N = 13, male N = 5; GN female N = 8, male N = 3; GG female N = 14, male N = 1). I also searched for fresh nests on each of these collecting trips and continued to make weekly trips (N = 4) after I finished collecting

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adult lizards until I found fresh nests at each site. In this way, I was able to determine when oviposition began at each site.

Lizards were transported to the University of Sydney where they were weighed and measured (SVL). Females were paired with a male (from the same population) and housed in cages (29.6 x 20.5 x 10.0 cm, L x W x H) until females were found to be gravid by abdominal palpation. Males were then removed and females were allowed to oviposit. Males were released at their sites of origin. Because our sample of males was limited, it was not possible to pair all females with a male. In such cases, females were placed alone in cages that were identical to those housing pairs. Cages were provisioned with moist vermiculite, shelter items, and water in a dish *ad libitum*. Lizards were fed live crickets (*Acheta domesticus*, approximately 2.0 cm long) dusted with reptile vitamins every three days (more below). Cages were placed on 6 shelves in a room maintained on a 12-h light:12-h dark photoperiod to mimic natural conditions during the reproductive season. Temperature was regulated using heating elements beneath the cages. When on, the heating elements maintained a thermal gradient of 20-35°C within cages, whereas temperatures fell to ambient room temperature (17 ± 1°C) within cages when heating elements were off (more below). I inspected all cages for eggs twice daily.

One-half of the females from each site were randomly assigned to "hot" or "cold" temperature treatments. For

hot and cold treatments, heating elements remained on 8 h/d and 3 h/d respectively (Fig 2). Temperature treatments were designed to mimic natural conditions at low and high elevations. Cages were rotated along shelf rows every three days and among shelves each week to control for position effects. Only females that oviposited and were exposed to these treatments at least 10 d prior to oviposition were included in analyses (total N = 28, hot treatment N = 18, cold treatment N = 10, $\bar{x}$ d in thermal treatments ± 1.0 SE = 31.4 ± 2.6). There were no significant differences in mean SVL (AVOVA, $F_{1,26}$ = 0.89, P = 0.36) or body mass (ANOVA, $F_{1,26}$ = 0.57, P = 0.46) between females in the two thermal treatments.

To quantify temperature differences between "hot" and "cold" treatments, 12 post-oviposition females (6 from each treatment, 2 from each shelf) were fitted with thermochron data loggers set to record temperature every 10 minutes for 10 days. Casings were removed from the data loggers (Robert and Thompson 2003) to reduce their mass (1.5 g). Thermochrons were superglued onto the dorsal surface of lizards, just above the pectoral girdle. Thermochrons did not hinder lizard movements, and body-surface temperatures recorded this way correlate highly with internal body temperatures (Robert and Thompson 2003). Lizards were monitored twice daily and for those that had dislodged their thermochrons, the devices were reapplied. I analysed data

from 5 days where thermochrons remained affixed to the lizards for entire 24-h periods (midnight – midnight). From these data, I computed mean daily, mean minimum, and mean maximum temperatures for each female. Because these data were non-normally distributed and could not be satisfactorily transformed, I used a Mann-Whitney test to examine differences in mean, minimum, and maximum temperatures experienced by females within the two treatments. After oviposition and thermal monitoring, all females were returned unharmed to their respective collection sites.

   To determine the effect of thermal treatments on food intake, I recorded the number of crickets consumed by 29 of the lizards (*N* = 6 male, 23 female) for 30 d prior to the beginning of oviposition. Every three days, the number of uneaten crickets (live or dead) remaining in each cage was recorded. Dead crickets were removed and the number of live crickets in each cage was increased to 15 per individual (30 in cages with pairs). This ensured that the opportunity to forage remained constant for each individual. I calculated mean daily food intake (number of crickets/d) for each lizard. I used ANCOVA to analyse the effects of temperature treatment and site of origin on food intake, with SVL at the time of capture as the covariate. Outside the 30-d period prior to oviposition, lizards were fed ~ 15 crickets every three days.

As soon as I discovered eggs (Fig 1), the entire clutch was removed and both eggs and females were weighed using an electronic balance (± 0.001 g). One egg from each clutch was preserved in 70% ethanol and, approximately 1 month later, dissected for embryonic staging. I determined the embryonic stage of development of eggs using methods and embryonic staging criteria developed for reptiles (Dufaure and Hubert 1961; as modified by Muthukkaruppan et al. 1970; Shanbhag et al. 2001 for oviparous lizards). Because I was unable to confidently stage 6 eggs (hot treatment N = 3, cold treatment N = 3), I removed these from analyses. I assumed for analyses that eggs within clutches did not vary in stage of development at oviposition: such constancy has been demonstrated for other lizards (Muthukkaruppan et al. 1970; Mathies and Andrews 1995), but has not been examined in *B. duperreyi*. I used two-factor ANOVA to examine the effects of maternal thermal treatment and site of origin on embryonic stage at oviposition ($\bar{x}$ individuals per group ± 1.0 *SE* = 3.7 ± 0.92), and linear regression to examine the effects of embryonic stage at oviposition on mean egg mass and mean incubation duration (later split by thermal treatment).

Nest temperatures at GN and GG did not differ (see below). Therefore I re-grouped individuals into "high elevation" (GN and GG) and "low elevation" (PC) groups for the following statistical analyses. Neither mean female

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SVL nor body mass (ANOVA: $F_{1,26}$ = 0.29, P = 0.60 and $F_{1,26}$ = 0.77, P = 0.41, respectively) differed significantly between the high and low elevation groups. With individual females as experimental units, I used two-factor ANOVA to examine the effects of maternal thermal treatment and elevation on date of oviposition (in the laboratory, calculated as number of days from 1 Nov), mean egg mass, number of eggs per clutch, and total clutch mass. I included SVL as a covariant when analysing egg mass and number of eggs per clutch, and included post-oviposition body mass as a covariant when analysing total clutch mass. I used simple linear regression to examine the effects of foraging rate (see above) on mean egg mass and the number of eggs produced per clutch, and multiple regression to analyse the effects of foraging rate on total clutch mass, with post-oviposition female body mass as the additional covariate. Data for two females were removed from the egg and clutch mass analyses because one or more of their eggs showed signs of desiccation at collection.

Egg Experiments

After removal of eggs for embryonic staging (above) and of six unfertilized eggs, the remainder (N = 126 eggs) were placed in individual 64-mL glass jars containing moist vermiculite (water potential −200 kPa) and sealed with plastic food wrap to prevent evaporation (Shine and Harlow

1996). Eggs from each clutch were selected randomly and placed inside cycling-temperature incubators with sinusoidal diurnal thermal cycles set to mimic natural nest conditions at low and high elevations (low elevation "hot" treatment = 22 ± 7.5°C; high elevation "cold" treatment = 16°C ± 7.5°C) such that each incubator contained half of the eggs from each clutch. Incubator shelves were rotated each week to control for possible position effects. I was not able to replicate incubators at each temperature regime, but the extent of phenotypic variation induced by differences among incubators is very small relative to thermal effects (Flatt et al. 2001).

Eggs were checked daily for hatching, and hatchlings were measured (SVL, tail length, mass, head length). I measured head length using electronic callipers (± 0.01 mm) as the distance from the anterior edge of the tympanum to the tip of the snout (Wymann and Whiting 2002). Husbandry for hatchlings was identical to that of gravid females in the "hot" treatment. At 7 days of age, I measured hatchling locomotor ability using a 1-m raceway with photocells at 25 cm intervals maintained at 24 ± 1.0° C (see Elphick 1995). Hatchlings were allowed to equilibrate to room temperature for 30 min, and then placed at one end of the raceway. An artist's paintbrush was used to "chase" the lizard, and I recorded the time it took to cover each 25-cm interval. Each lizard was raced three times with a minimum of 15 min rest between successive runs. For

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analysis, I examined mean speeds (over 1 m) and sprint speeds (over the fastest 25 cm). The lizards were then re-measured (SVL, tail length, mass).

With eggs/hatchlings as experimental units, I used multi-factor ANOVA to examine the effects of elevation, maternal thermal treatment, and incubation thermal treatment on survival of eggs to hatching (dichotomous dependent variable, Lunney 1970: 1 = hatched, 0 = failed to hatch). Because my data on incubation duration failed to meet the assumptions for parametric statistics despite transformation, I used a Komolgorov-Smirnov analysis to test for effects of the three factors described above on incubation duration. To examine the effects of elevation, maternal thermal treatment, and incubation thermal treatment on hatchling phenotype, I used a multivariate analysis of variance (MANOVA). The dependent variables in this analysis were neonate SVL, tail length and body mass at hatching and at one week of age, head length at hatching, mean 1-m running speed, and mean fastest 25-cm running speed.

RESULTS

Temperatures Within Natural Nests

Temperatures within natural *B. duperreyi* nests varied by elevation ($F_{2,115}$ = 47.03, P < 0.0001), with nests from GN and GG (high elevation sites) being similar to each other (Fisher's PLSD, P = 0.11) and 2.7°C cooler, on average, than

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PC nests (Fisher's PLSD, $P < 0.0001$; Fig 2A). Additionally,

mean nest temperatures at PC varied from year to year ($F_{6,90}$

$= 10.22$, $p < 0.0001$, Fig 2B). This variation resulted from

weather conditions, rather than among-year shifts in the

types or sizes of rocks under which eggs were laid, because

nests found under the same cover rock each year at PC

displayed similar thermal variation to that of all PC nests

in those years (Fig 2B).

Laboratory Thermal Treatment

Daily mean temperatures experienced by females in the

hot and cold laboratory thermal treatments differed by 1.80

°C ($U_{1,10} = 0.0$, $U'_{1,10} = 30.0$, $P = 0.0062$, Fig 3). By

contrast, minimum and maximum temperatures did not differ

($U_{1,10} = 13.0$, $U'_{1,10} = 17.0$, $P = 0.72$, and $U_{1,10} = 9.0$, $U'_{1,10} =$

21.5, $P = 0.27$ respectively, Fig 3). The slow post-basking

decline in body temperatures of "cold"-treatment females

(Fig 3) reflects heating of the entire room by the heat

sources for "hot" treatment cages.
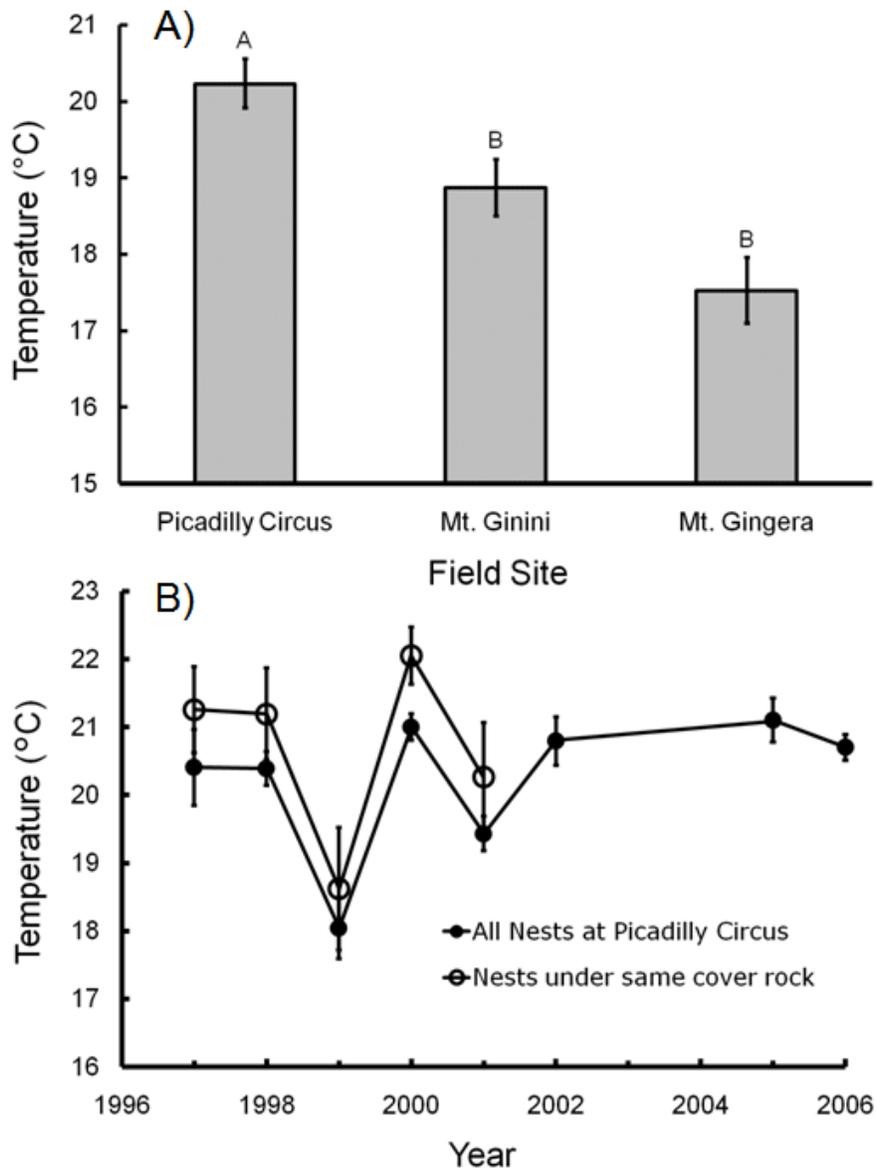
Figure 2. Temperature variation within natural nests of the lizard *Bassiana duperreyi*: a) grand mean (± 1.0 *SE*) temperatures at Picadilly Circus (1240 m asl), Mt. Ginini (1615 m asl), and Mt. Gingera (1670 m asl), different letters over adjacent bars indicate statistically significant ($P < 0.05$) differences; b) mean (± 1.0 *SE*) annual temperatures from 1997 – 2006 within all nests at Picadilly Circus and within nests located under the same cover rock at Picadilly Circus in successive years.
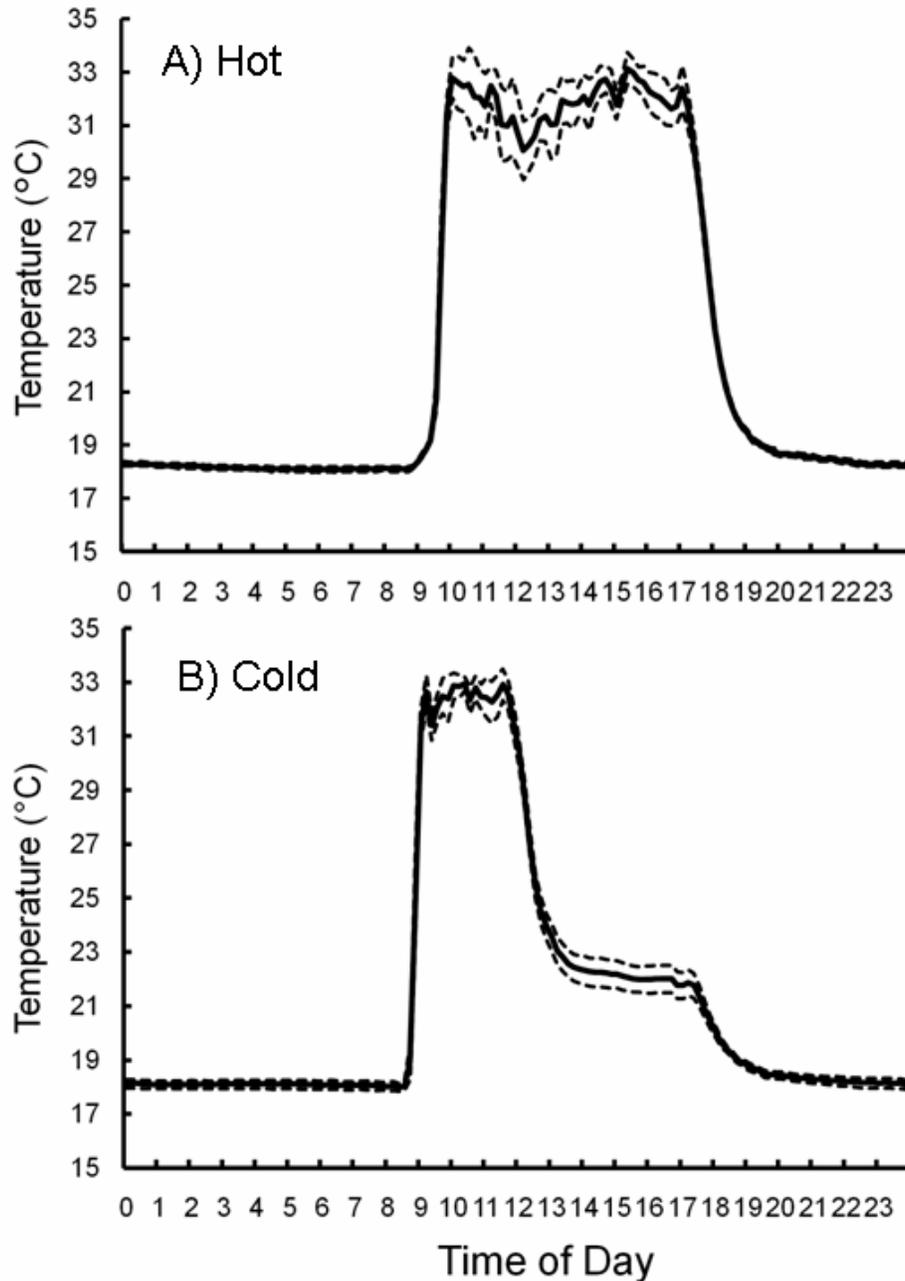
Figure 3. Laboratory thermal regimes over a 24-h period for a) hot (under cage heating elements switched on 8 hrs/d), and b) cold (under cage heating elements switched on 3 hrs/d) treatments. Five day means of records from thermal data loggers affixed to 11 post-oviposition female lizards (cold treatment N = 6, hot treatment N = 5) are presented. The dashed lines are ± 1.0 SE, of mean temperatures.

Influence of Temperature on Female Reproduction

The developmental stage of embryos produced by *B. duperreyi* at oviposition ranged from 30.5 (produced by 2 females in the hot treatment, one from GN and one from PC) to 33.5 (produced by a female in the cold treatment from GN), and was not significantly linked to mean egg mass ($F_{1,18}$ = 0.37, r^2 = 0.02, P = 0.55) or incubation duration (cold treatment: $F_{1,14}$ = 1.03, r^2 = 0.07, P = 0.33; hot treatment: $F_{1,18}$ = 0.26, r^2 = 0.01, P = 0.61). Site of origin affected embryonic stage at oviposition ($F_{2,16}$ = 11.91, P = 0.0007), such that females from high-elevation populations produced eggs with embryos further developed than those of females from the low-elevation population (Fig 4). Furthermore, females (particularly from GN) exposed to cold conditions in the laboratory oviposited eggs at later ($F_{1,16}$ = 14.62, P = 0.0015) embryonic stages of development than those produced by females exposed to hot laboratory conditions (Fig 4), with a significant interaction ($F_{2,16}$ = 5.07, P = 0.020) between the two factors.

I first discovered nests with eggs on 27 November at the low elevation site (PC), but not until 18 December at either high elevation site (GN and GG; 21-d difference). Nonetheless, elevation of origin did not affect date of oviposition in the laboratory ($F_{1,25}$ = 1.44E −6, P = 0.999; Fig 5A). By contrast, maternal thermal treatment affected

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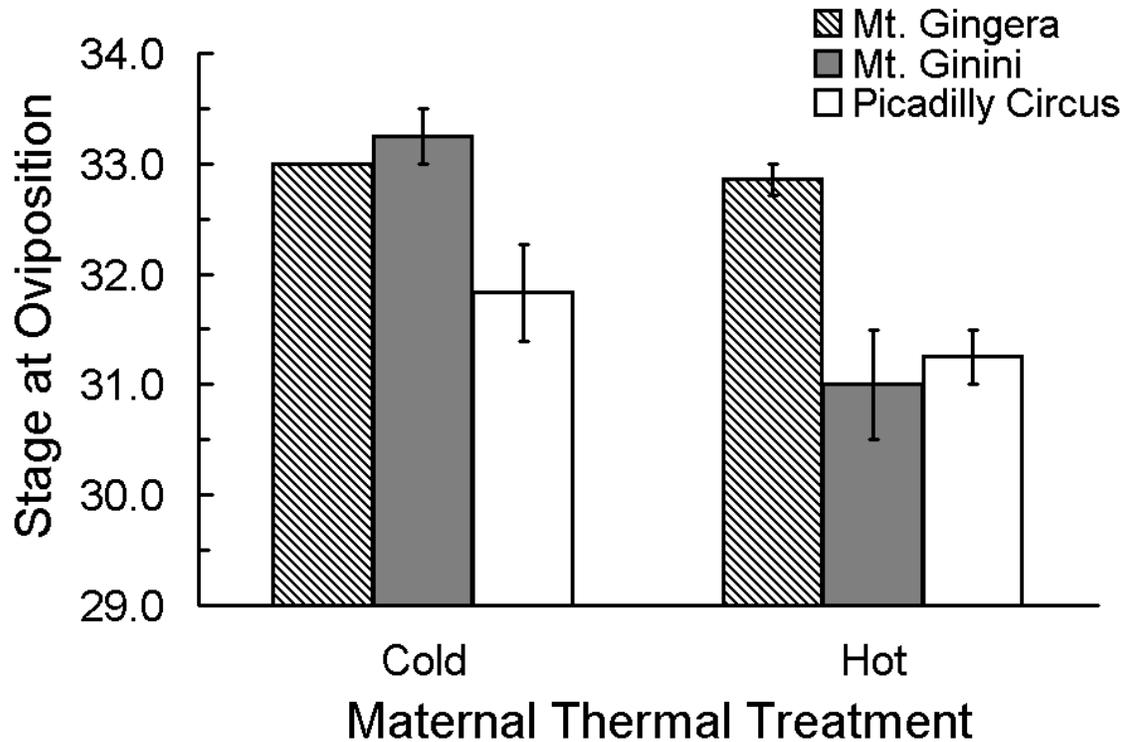
Figure 4. Effect of laboratory thermal conditions and site of origin on the developmental stage of embryos ($\bar{x} \pm 1.0\ SE$) produced by the lizard *Bassiana duperreyi* at oviposition.

laboratory oviposition date ($F_{1,25} = 21.08$, $P = 0.0001$); females exposed to cold temperatures oviposited later than did females exposed to warm temperatures (Fig 5A).

On average, each *B. duperreyi* consumed 3.91 crickets per day (1.0 SE = 0.1) over the 30-d period. Daily food intake increased with lizard SVL ($F_{1,24} = 4.45$, $P = 0.045$), but was not affected by thermal treatment ($F_{1,24} = 0.05$, $P = 0.83$) or site of origin ($F_{2,24} = 0.17$, $P = 0.85$). None of my measures of maternal investment (clutch size, egg mass,

total clutch mass) were affected by elevation or maternal

thermal treatment (Table 1). Maternal SVL positively

affected clutch size ($F_{1,24}$ = 16.78, P = 0.0004), but did not

affect mean egg mass ($F_{1,22}$ = 0.02, P = 0.90). Post-

oviposition body mass had a nearly significant affect on

clutch mass ($F_{1,21}$ = 3.78, P = 0.07). Moreover, food intake

did not correlate with any of the "maternal investment"

traits that I measured (clutch size: $F_{1,15}$ = 3.27, r^2 = 0.18,

P = 0.09; egg mass: $F_{1,13}$ = 0.22, r^2 = 0.02, P = 0.65; clutch

mass: $F_{2,12}$ = 2.66, r^2 = 0.31, P = 0.11).

Influence of Temperature on Hatchling Survivorship and

Phenotype

   Of the 126 *B. duperreyi* eggs placed in incubators, 96

(76.2 %) hatched. Egg survivorship was increased by higher

temperatures during both pre-oviposition ($F_{1,122}$ = 4.57, P =

0.03) and post-oviposition ($F_{1,122}$ = 4.36, P = 0.04) periods,

but was not affected by elevation ($F_{1,122}$ = 0.91, P = 0.34,

Figs 5C-5D). Only temperature during incubation (χ^2 =

93.32, df = 2, P < 0.0001) affected incubation duration

(maternal thermal regime: χ^2 = 1.85, df = 2, P = 0.79;

elevation: χ^2 = 1.57, df = 2, P = 0.91) with eggs kept at 16

± 7.5 ºC incubating for longer than eggs kept at 22 ± 7.5 ºC

(Fig 5B).

Table 1: Maternal investment by female lizards *Bassiana duperreyi* collected from high and low elevations, and exposed to hot and cold thermal treatments in the laboratory prior to laying. Data are F-statistics derived from two-factor ANOVA and means ± 1.0 *SE*.

Variables	Elevation		Maternal Treatment	
	Low	High	Hot	Cold
Eggs/Clutch	$F_{1,24} < 0.01$		$F_{1,24} = 0.03$	
	5.5 ± 0.72	5.8 ± 0.51	6.9 ± 0.48	6.3 ± 0.78
Egg Mass (g)	$F_{1,22} = 0.21$		$F_{1,22} = 0.32$	
	0.36 ± 0.01	0.35 ± 0.01	0.36 ± 0.01	0.34 ± 0.01
Clutch Mass (g)	$F_{1,21} = 0.14$		$F_{1,21} = 0.68$	
	1.99 ± 0.28	2.11 ± 0.22	2.20 ± 0.22	1.84 ± 0.26

MANOVA revealed that maternal thermal treatment (Pillai's Trace = 0.52, $F_{9,40} = 4.78$, $P < 0.001$), incubation thermal treatment (Pillai's Trace = 0.71, $F_{9,40} = 10.97$, $P < 0.001$), and (to a marginal extent) elevation (Pillai's Trace = 0.32, $F_{9,40} = 2.11$, $P = 0.051$) all affected hatchling phenotypes in *B. duperreyi* (Fig 5, Table 2). The only significant interaction (Pillai's Trace = 0.36, $F_{9,40} = 2.53$, $P = 0.021$) was between maternal thermal treatment and elevation. At hatching, SVL was positively affected by the

temperatures experienced by eggs pre-oviposition ($F_{1,48}$ = 9.54, P = 0.003, Fig 5E) and post-oviposition ($F_{1,48}$ = 11.18, P = 0.002, Fig 5F). However, hatchlings from high-elevation parents were larger (SVL - $F_{1,48}$ = 5.99, P = 0.02) than hatchlings from low elevation populations (Figs 5E–5F). This pattern persisted for at least 7 days, although the effect of maternal thermal treatment diminished (Table 2). Interestingly, effects of maternal thermal treatment and elevation on tail length were not apparent at hatching but appeared within 7 days (Table 2). Hatchling body mass was affected by maternal thermal treatment but this effect did not persist (Table 2). The only effect I found approaching significance on hatchling performance was that higher incubation temperature increased 25-cm sprint speed (Table 2).

Table 2. Effects of elevation, maternal thermal treatment, and incubation thermal treatment on the phenotypes of *Bassiana duperreyi* at hatching (0d) and 1 wk of age (7d). "SVL" denotes snout-vent length, "TL" denotes tail length, "HL" denotes head length, and "1-m" and "25-cm" denote running speed over those distances. Data are F-statistics derived from separate three-factor ANOVA's (*df* = 1,40) and $\bar{x}$ ± 1.0 *SE* (*SE* of 0.00 indicates an *SE* < 0.005). For interactions, "E" denotes elevation, "M" maternal thermal treatment, and "I" incubation thermal treatment. Bold face indicates significant (*P* < 0.05) differences whereas italics indicate differences approaching significance (0.07 > *P* ≥ 0.05).

Variables	Elevation		Maternal Treatment		Incubation Treatment		Interactions
	Low	High	Hot	Cold	Hot	Cold	
SVL 0d(mm)	*F = 5.992*		*F = 9.538*		*F = 11.184*		*E + I, E + M*
	28.2 ± 0.32	**28.6 ± 0.25**	**28.6 ± 0.23**	**28.0 ± 0.35**	**29.4 ± 0.18**	**27.3 ± 0.29**	
SVL 7d(mm)	*F = 4.174*		*F = 3.520*		*F = 15.191*		None
	29.5 ± 0.27	**29.9 ± 0.21**	*30.0 ± 0.18*	*29.2 ± 0.37*	**30.3 ± 0.17**	**28.5 ± 0.20**	
TL 0d (mm)	*F* = 0.025		*F* = 0.240		*F = 29.889*		None
	30.7 ± 0.43	30.8 ± 0.34	30.9 ± 0.31	30.3 ± 0.51	**32.5 ± 0.28**	**28.7 ± 0.22**	

Variables	Elevation		Maternal Treatment		Incubation Treatment		Interactions
	Low	High	Hot	Cold	Hot	Cold	
TL 7d (mm)	**F = 7.760**		**F = 11.318**		**F = 31.519**		**E + I**, *E + M*
	32.0 ± 1.16	**31.3 ± 1.09**	**32.3 ± 0.85**	**29.4 ± 1.94**	**32.8 ± 1.03**	**28.9 ± 1.20**	
BM 0d (g)	F = 0.736		**F = 5.928**		F = 0.004		None
	0.30 ± 0.00	0.31 ± 0.00	**0.31 ± 0.00**	**0.28 ± 0.01**	0.31 ± 0.01	0.30 ± 0.00	
BM 7d (g)	F = 1.314		F = 0.978		F = 0.155		None
	0.28 ± 0.01	0.29 ± 0.01	0.29 ± 0.00	0.27 ± 0.01	0.29 ± 0.00	0.28 ± 0.01	
HL 0d (g)	F = 1.698		F = 1.028		F = 0.155		None
	5.3 ± 0.05	5.3 ± 0.03	5.3 ± 0.03	5.3 ± 0.06	5.3 ± 0.03	5.3 ± 0.05	
1-m (m/s)	F = 1.906		F = 0.648		F = 2.442		None
	0.36 ± 0.01	0.34 ± 0.01	0.35 ± 0.01	0.35 ± 0.02	0.36 ± 0.01	0.33 ± 0.02	
25-cm (m/s)	F = 0.227		F = 0.684		*F = 3.531*		None
	0.45 ± 0.02	0.43 ± 0.02	0.44 ± 0.02	0.43 ± 0.02	*0.46 ± 0.02*	*0.40 ± 0.02*	

Table 2 Cont.
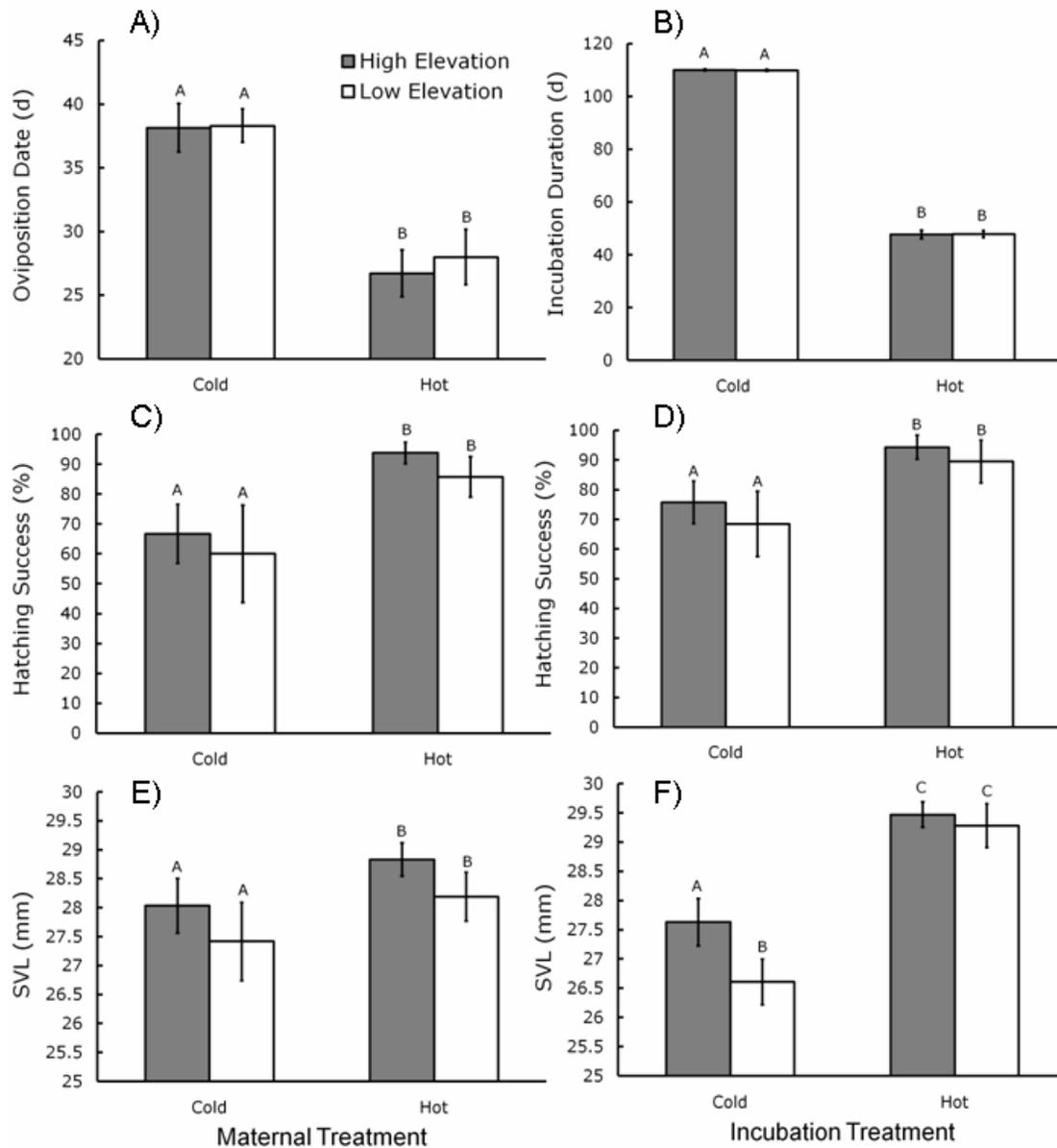
Figure 5. Thermal treatment (maternal and incubation) and elevation of origin effects on reproductive parameters in the lizard *Bassiana duperreyi* ($\bar{x}$ ± 1.0 *SE*): a) date of oviposition (calculated as d from 1 Nov.), b) incubation duration, c) - d) survival of eggs to hatching, and e) - f) snout-vent length (SVL) at hatching. Different letters above adjacent bars indicate statistically significant (*P* < 0.05) differences.

DISCUSSION

Geographic variation in reproductive traits is widespread in lizards (e.g., Smith et al. 2003; Du et al. 2005; Doody et al. 2006), as in many other types of animals (e.g., flatworms: Ducey et al. 2005; primates: Lahann et al. 2006; fish: Licandeo and Cerna 2007; and insects: Tanaka and Zhu 2008), and can have many explanations (Shine 2005). One of the most common involves geographic variation in food availability, and thus the resources that females can allocate to reproduction (Sears 2005; Shine 2005; Warner et al. 2007). However, I found no difference in either food intake or maternal investment by *B. duperreyi* in my treatments, suggesting that the nutritional environment is not likely responsible for my results. Instead, thermal factors appear most important. Many authors have suggested that cold temperatures stimulate the evolution of viviparity via intermediate stages of progressively increasing durations of oviductal retention of developing embryos (e.g. Blackburn 1982; Shine 1985; Andrews et al. 1997). My data support this scenario, provide intraspecific evidence of the predicted shift, and clarify the proximate mechanism generating a shift towards viviparous reproduction in colder areas.

At higher elevations, female *Bassiana duperreyi* retain their eggs *in utero* for longer than is the case at lower elevations, and therefore complete a greater proportion of

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embryogenesis prior to oviposition. Thus, high-elevation

females are somewhat further towards the "viviparous" end of

the oviparity-viviparity continuum than are lower-elevation

conspecifics. This result is consistent with both theory

(Shine and Bull 1979; Shine 1985, 1995) and the higher

incidence of viviparous squamate taxa in cooler climates

(Weekes 1935; Shine 1985). Results from my experimental

manipulation of maternal thermoregulatory opportunities

suggest that the elevational shift in degree of uterine

retention is partially canalised (i.e., under identical

thermal regimes, high-elevation females retain eggs for

longer) but is also phenotypically plastic (i.e., exposure

to cool conditions directly stimulates prolongation of

uterine retention: Fig 4). Underlying reaction norms for

uterine retention of eggs under cool conditions thus may

diverge between populations (Fig 4). The end result is that

the more advanced stage of embryogenesis at oviposition in

higher-elevation lizards appears to be a result both of

"traditional" (canalised) adaptation and of phenotypic

plasticity. It remains possible, however, that the

"canalised" divergence between populations reflects longer-

term or earlier-acting phenotypic plasticity: that is,

prolonged uterine retention of eggs by high-elevation

females might be a facultative response to low temperatures

experienced prior to ovulation, possibly even early in the

female's life (e.g., during her own period of

embryogenesis). Long-term experimental studies are needed to tease apart such possibilities.

A combination of adaptation and plasticity to match reproductive modes to thermal environments would accord well with theoretical models. Theory predicts that canalised traits enhance organismal fitness when fitness challenges (and thus, optimal phenotypes) are consistent through time, whereas facultative (plastic, environmentally-induced) responses are better suited to challenges that vary unpredictably through time (Bull 1980; Scharloo 1989; Brommer et al. 2008). Thermal regimes in nest sites of *Bassiana duperreyi* exhibit both forms of variation: higher-elevation sites are consistently cooler (Fig 2A) but the exact thermal regimes (and hence, presumably, the selective advantages of uterine retention) vary from year to year in an apparently unpredictable fashion (Fig 2). Modelling suggests that the optimal response to such a situation is to evolve reaction norms (cooler temperatures elicit a delay in oviposition, with more embryogenesis completed prior to laying), but with the specific trait values (degree of embryogenesis elicited by any specific thermal regime) varying between sites that differ consistently in mean thermal regimes. Site-specific adaptation (i.e., canalisation) of traits also may be precluded by gene flow among adjacent populations (Niewiarowski and Roosenburg 1993; Sears and Angilletta 2003; Butlin et al. 2008).

Because it takes approximately three days for lizard embryos to pass through each of the three modal stages (Muthukkaruppan et al. 1970) that I observed, the variation in development in my study equates to approximately nine days. However, my data suggest that this variation had no effect on incubation duration, which is inconsistent with the argument that oviductal egg retention at high maternal body temperatures enhances offspring fitness by accelerating embryogenesis and thus hatching (Packard et al. 1977; Blackburn 1982; Shine 1985). A more likely adaptive advantage of egg retention by *B duperreyi* in cold conditions is that eggs retained *in utero* produce fitter offspring than do eggs that are oviposited at earlier embryonic stages (maternal manipulation hypothesis, Shine 1995; Webb et al. 2006; Radder et al. 2008). Because thermal effects on offspring phenotype are stronger early in incubation than later (Shine 2005; Brana and Ji 2007; Radder et al. 2008), retention of eggs, even through relatively few stages of development as in *B. duperreyi*, may strongly impact offspring phenotype. My result that thermal regimes affect offspring fitness both before and after oviposition in *B. duperreyi*, with warmer conditions in both phases resulting in higher survival rates (hatching success) and apparently fitter (larger, faster) hatchlings support this hypothesis.

Embryo retention in *B duperreyi* likely illustrates an important first step in the evolution of viviparity, with

further steps in that transition favoured under circumstances that confer even higher benefits to uterine retention (e.g., lowered availability of warm nest sites) or reduce the costs of prolonged retention to females (e.g., vulnerability to predation while heavily burdened with eggs: Shine 2005). Most often, however, further costs disfavour prolongation of uterine retention. For example, there may be a trade-off between egg shell thickness and the gas exchange demands of developing embryos (Thompson and Stewert 1997; Andrews and Mathies 2000; Shine and Thompson 2006). Gas exchange can be increased by reductions in eggshell thickness, but at the cost of increased permeability to water (Shine and Thompson 2006). Female *B. duperreyi* in cold environments may only retain eggs through a few extra developmental stages because further egg retention would require eggshell reduction, and hence confer other costs.

Although warmer thermal treatments in the laboratory resulted in larger hatchlings, elevation had the opposite effect: hatchlings from high-elevation (cold) populations were larger than offspring of low elevation (warmer) populations. This interpopulation pattern in *B. duperreyi* follows Bergmann's rule (tendency for larger individuals at cooler temperatures, Blackburn et al. 1999; Sears and Angilletta 2004). Although MANOVA revealed a significant interaction between elevation and maternal thermal treatment, the effects of elevation on hatchling phenotype were minor compared with the effects of incubation

temperature. This disparity suggests that plastic responses to the local thermal environment may generate much of the phenotypic variation among these populations, and in particular the trend for females in higher-elevation populations to retain their eggs longer, and hence more closely approach viviparity.

CONCLUSIONS

My data provide much needed intraspecific evidence for the hypothesis that cold climates select for viviparity in squamate reptiles. Variation in egg retention by *B. duperreyi* appears to be both partially canalized among populations and phenotypically plastic. In fact, mine is the first study that I am aware of showing that embryonic stage at oviposition fluctuates plastically in response to thermal conditions. This pattern of variation is predicted to evolve when interpopulation temperatures vary consistently but interannual temperatures vary unpredictably, as my data suggest happens within natural *B duperreyi* nests in the Brindabella Ranges. Moreover, my data suggest that warmer temperatures eggs are exposed to both before and after oviposition increase offspring fitness, but embryonic stage at oviposition does not affect hatching date in *B duperreyi*. These data support the hypothesis that fitter offspring resulting from increased temperatures *in utero*, rather than earlier hatchling

emergence, select for egg retention in this species. In conclusion, *B. duperreyi* display how subtle changes toward increased egg retention are adaptive in cold environments, and therefore may be a model for the initial step towards the evolution of viviparity in squamates.

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