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ALTITUDINAL DIVERGENCE IN MATERNAL THERMOREGULATORY BEHAVIOUR MAY BE DRIVEN BY DIFFERENCES IN SELECTION ON OFFSPRING SURVIVAL IN A VIVIPAROUS LIZARD

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Plastic responses to temperature during embryonic development are common in ectotherms, but their evolutionary relevance is poorly understood. Using a combination of field and laboratory approaches, we demonstrate altitudinal divergence in the strength of effects of maternal thermal opportunity on offspring birth date and body mass in a live-bearing lizard (*Niveoscincus ocellatus*). Poor thermal opportunity decreased birth weight at low altitudes where selection on body mass was negligible. In contrast, there was no effect of maternal thermal opportunity on body mass at high altitudes where natural selection favored heavy offspring. The weaker effect of poor maternal thermal opportunity on offspring development at high altitude was accompanied by a more active thermoregulation and higher body temperature in highland females. This may suggest that passive effects of temperature on embryonic development have resulted in evolution of adaptive behavioral compensation for poor thermal opportunity at high altitudes, but that direct effects of maternal thermal environment are maintained at low altitudes because they are not selected against. More generally, we suggest that phenotypic effects of maternal thermal opportunity or incubation temperature in reptiles will most commonly reflect weak selection for canalization or selection on maternal strategies rather than adaptive plasticity to match postnatal environments.

KEY WORDS: Life-history evolution, maternal effect, phenotypic plasticity, selection—natural.

Temperature is one of the most important abiotic factors that restricts species distributions and causes ecological and evolutionary diversification (Angilletta 2009). All life stages are potentially affected by temperature but ontogenetic stages of extensive growth and differentiation should be particularly sensitive. Indeed, the thermal conditions experienced during embryonic development have been shown to affect birth or hatching date and several morphological, physiological, and behavioral traits of offspring in many ectotherms, such as reptiles (e.g., Atkinson 1996; Deeming 2004; Booth 2006). Such effects are usually interpreted as either passive consequences of temperature on physiological processes with negative or neutral consequences on fitness (Atkinson 1996; de Jong and van der Have 2009) or, alternatively, as adaptive responses to match phenotypic traits to posthatching environmental conditions (reviewed in Shine 2004; Gibbs and van Dyck 2009). However, our ability to make inferences about the evolutionary implications of thermal effects on offspring phenotype is limited for several reasons. First, phenotypic effects of temperature show variation across species and it is unclear whether particular responses are consistent and repeatable within and between populations or environments (reviewed in Deeming 2004). Second, the fitness consequences of temperature-induced plasticity in natural populations remain largely unexplored (Brown and Shine 2004; Buckley et al. 2007; Calsbeek and Sinervo 2007; Wapstra et al. 2010). Third, maternal nest site choice or thermoregulatory behavior can mediate how local abiotic conditions translate into variation in thermal conditions experienced by developing embryos (Shine 1995, 2004; Resetarits 1996; Webb et al. 2006; Telemeco et al. 2009). Thus, maternal behavior may not only contribute to the patterns of phenotypic variation in natural populations, but also enhance or reduce the scope for natural variation in temperature to generate evolutionary change. However, geographic variation in nest site choice or behavioral thermoregulation and their repeatability and heritability have rarely been studied (Resetarits 1996; Janzen and Morjan 2001; Doody et al. 2006; Warner and Shine 2008; Angilletta et al. 2009). Thus, we are poorly equipped to assess the adaptive significance of observed patterns of developmental plasticity in response to temperature.

Here, we use experimental and correlative data from populations of a viviparous lizard (*Niveoscincus ocellatus*) at the climatic extremes of the species' distribution to test: (1) whether the effects of maternal thermal opportunity on the timing of birth and offspring mass are consistent among years and populations; (2) whether maternal behavior is consistent with how variation in the maternal thermal opportunity translates into phenotypic variation in offspring within and between populations; (3) the consequences of this variation for offspring survival; and (4) the genetic basis (heritability) of birth date and offspring mass in natural populations. Our results show that lowland and highland populations have diverged with respect to how maternal thermal opportunity affects offspring development. Consistent selection favoring high offspring body mass in highland populations was associated with more opportunistic female thermoregulatory behavior, in particular in poor thermal conditions. This generates a higher and more stable gestation temperature and may buffer offspring from variation in the external thermal conditions. On the contrary, at low altitude, where offspring mass was strongly affected by maternal thermal opportunity, we did not find ongoing selection on offspring body mass. We discuss the implications of our results for the evolution of phenotypic plasticity in response to incubation temperature in reptiles and how such effects could be interpreted in terms of past patterns of selection.

Methods

Niveoscincus ocellatus is a small skink that occurs over a 1200 m altitudinal, and climatic, gradient from sea level to alpine regions throughout Tasmania, Australia. Life-history traits vary accordingly, with skinks at higher altitudes emerging from hibernation later in the year, having a shorter activity period, larger body size, and increased clutch size and offspring size than skinks at low altitudes (Wapstra et al. 1999, 2001; Wapstra and Swain 2001; Cadby et al. 2010). Here, we use data from experimental manipulation of basking conditions (4 h or 10 h basking opportunity per 24 h) replicated across four years for females from the extremes of the altitudinal distribution of the species. Furthermore, we analyze data from nine years of long-term studies of two populations at these altitudes to establish how body mass and birth date vary in the wild, and, in three of those years, to generate estimates of offspring survival.

EXPERIMENTAL PROCEDURES

We manipulated thermal conditions during gestation in a common garden experiment, using females from highland (> 1000 m altitude) and lowland (< 200 m altitude) populations, replicated in four years; 2000, 2006, 2007, and 2008. The location of sampling of females varied between years to ensure that no females were ever used twice, but populations within each altitude (including those subject to long-term studies; see below) occur within a 150 m altitude range and experience the same climatic conditions. The experimental manipulation of basking regime followed the same basic protocol in each year. Briefly, females were brought into the laboratory (ambient temperature 16°C ± 1°C) at the University of Tasmania during the first half of gestation. They were housed in plastic terraria (30 × 20 × 20 cm) each containing cover and a basking light to provide a thermal gradient from 16°C to 35°C. Water was available ad libitum and lizards were fed three times per week on live insects and crushed fruit. A few days after capture, the females were randomly assigned to two treatment groups representing different thermal regimes. Each regime allowed females to bask to their preferred body temperature for a different length of time per day (i.e., when basking lights were on); either 10 h per day (high opportunity for thermoregulation), or 4 h per day (low opportunity for thermoregulation). These conditions reflect natural annual variation in thermal opportunities for *N. ocellatus* at these sites (Wapstra et al. 1999; Wapstra 2000) and result in birth dates that span natural birth dates (Wapstra 2000; Wapstra et al. 2010; see Results). Overhead lights (14:10 h light:dark photoperiod) provided identical day lengths for both treatments, reflecting natural day lengths during summer. All lizards were maintained under these conditions until parturition, with the terraria positioned randomly with regard to the experimental treatment, and repositioned fortnightly to minimize positional effects. At the end of gestation, terraria were checked twice daily for neonates. At birth, offspring were measured (mass: ± 0.1 mg, snout-vent length: ± 0.01 mm), sexed by hemipene eversion (Harlow 1996), and presence of aborted or stillborn offspring was noted. All hatchlings were then released back into their population of origin.

Selected body temperatures

In the 2006 experimental replicate, female body temperature was monitored three times per week throughout gestation in both experimental treatments (high and low opportunity for thermoregulation). Body temperatures (± 0.1°C) of females were approximated by manually placing an infrared thermometer (Testo© 805 Infrarot-Thermometer) 1 cm above a female's back three times weekly (Hare et al. 2007). Sampling times varied between days but always occurred at least 1 h after basking lights were turned on. This allowed females time to reach their preferred body temperature. Variation in body temperature between individuals closely mirrors variation in basking behaviour (C. D. Cadby et al., unpubl. data).

Measures of basking behavior

In the 2008 experimental replicate, we conducted a detailed assessment of female basking behavior. Basking behavior for each individual was assessed at four times during gestation; at the beginning of the gestation (2 and 3 weeks after experimental assignment) and toward the end of gestation (6 and 7 weeks after experimental assignment). At each of these time periods, behavioral observations were taken in each of the two weeks, resulting in a total of four days of behavioral observations per individual. To assess basking behavior, the location of each individual female relative to the basking light was recorded every 15 min from 0745 h to 1815 h for the warm basking regime and from 0945 h to 1415 h for the cold basking regime. The position was recorded as either under the basking light or outside the range of the basking light (i.e., under the shelter or at the rear of the cage, which closely follows the temperature gradient). Behavioral monitoring did not cause disturbance to the animals, which displayed normal behavior throughout the study.

FIELD STUDIES

To assess the effect of thermal opportunity on offspring date of birth and offspring mass in the natural population, data were collected from two populations of individually marked animals (lowland population: 42°55′S, 147°87′E, elevation: 30 m; highland population: 41°86′S, 146°53′E, elevation: 1150 m, not used as source for experimental animals), also at the climatic extremes of the species' distribution. These populations have been monitored annually since 2000. At both populations, we captured most (∼ 90–95%) gravid female skinks at the end of gestation each year (late December at the lowland site and mid to late January for the highland site; well after the period when offspring development is sensitive to temperature; Neaves et al. 2006). Upon capture, females were identified individually using toe clips (or a new one was assigned) and their position recorded to ±5 m within the study site using a handheld global positioning system. Females were then returned to a laboratory at the University of Tasmania where they were held in identical conditions to those females held in the long thermal treatment (see above).

At birth, offspring were measured (mass: ±0.1 mg, snout-vent length: ±0.01 mm) and sexed by hemipene eversion as above. Sizes of adult females were similarly recorded (mass: ±0.1 mg, snout-vent length: ±1 mm). All offspring were then permanently marked by toe-clipping before they and their mothers were released back into the field population. Mothers were released at the original site of capture and offspring were released individually and randomly at one of 12 locations throughout the field site. Offspring were released at random locations within the study site to prevent interpretation of offspring growth and survival being compromised by some mothers occupying potentially advantageous microhabitats (Wapstra et al. 2010) and to enable more accurate heritability measures of target traits. Although strictly speaking this will disrupt a potential adaptive match between maternal and offspring environment, the potential for such highly local effects should be very minor given the dispersal of offspring and high variance in thermal profiles of habitat within populations.

Estimates of survival

We conducted extensive recaptures of all surviving first year offspring subsequent to emergence from hibernation (September–October) in three years (2000, 2001, and 2007). These recaptures measure offspring survival over the first 6 months of life, which represents a key period of offspring mortality in lizards (e.g., Svensson and Sinervo 2000; Lorenzon et al. 2001). At recapture, the date was recorded and the offspring were painted with a small dot to avoid unnecessary recapture. As an individual's average dispersal distance over its first year of life is low at both study populations (24 m ± 1.64 SE and 31 m ± 2.01 SE at the lowland and highland population, respectively) compared to the size of the study sites themselves (300 × 400 m and 100 × 800 m respectively) (E. Wapstra, unpubl. data) and >100 m outside the edges of the site are searched for offspring during each recapture, estimates of offspring survival are unlikely to be compromised by dispersal out of the study area. Recaptures were continued over a four-week period until only marked offspring were observed over several days of survey.

Climate data

We used daily maximum temperatures during the period of gestation (lowland: October 1 to January 1; highland: October 15 to January 15) as a measure of basking opportunity experienced by gravid female skinks in the field (Huey 1982; Wapstra et al. 2009). Maximum temperatures were recorded by the Tasmanian Bureau of Meteorology at meteorological stations situated near the lowland (Orford Meteorological Station) and the highland (Liawenee Meteorological Station) populations. The two regions differ considerably in their thermal regimes during the months corresponding to female gestation with a 50-year mean maximum temperature of 17.3°C, 18.6°C, and 20.4°C for October, November, and December, respectively for the lowland region compared to 11.9°C, 14.7°C, and 17.0°C for the same months in the highland region (Tasmanian Bureau of Meteorology). Activity patterns and basking opportunity vary accordingly (Wapstra et al. 1999).

STATISTICAL ANALYSES

We consistently used offspring mass rather than skeletal measures of size because mass is primarily determined during the latter part of gestation (when embryo size increases dramatically), whereas body proportions (and sex) can be sensitive to thermal conditions very early in development (even before oviposition in egg-laying species; Shine 2004) and therefore before we brought females into the laboratory. The effects of thermal treatment on birth date and offspring body mass were analyzed using linear mixed models with female identity (nested within treatment and population) as a random effect (PROC MIXED, SAS 9.2). Basking treatment, year, and population were entered as fixed effects and female snout-vent length (SVL) as a covariate. We also initially included offspring sex, but body mass at birth does not differ between males and females in this species (e.g., Wapstra et al. 2010) and there were no significant interactions between sex and other predictors and we therefore dropped it from further analyses. Repeatabilities of basking data from the laboratory as well as birth date (relative to the population average in each year) and offspring size from the wild were estimated from the intraclass correlation coefficient from repeated random effects models with female identity as the single factor (the same females were captured over multiple years; PROC MIXED, SAS 9.2). Heritability estimates from natural populations were obtained using mother–daughter regression based on averages for each mother and her offspring for both date of birth and offspring mass (Lynch and Walsh 1998). Population differences in basking behavior (estimated as the log-transformed proportion of the day spent under the basking light) in the 2008 experimental replicate were tested using a mixed model (PROC MIXED, SAS 9.2) with population and treatment and their interaction as fixed factors and female identity (nested within population and treatment) as a random effect.

Determinants of offspring survival were assessed using logistic models, corrected for overdispersion (Littell et al. 2006). We initially included maternal identity as a random effect despite that few females recruited more than one offspring (PROC GLIMMIX SAS 9.2; Littell et al. 2006), but because the variance explained was estimated to be zero and resulted in poor model fit for some models, we report results from models excluding female identity. Our initial analysis of patterns of survival included year, population, offspring sex and their interactions as fixed factors, and birth mass and birth date as covariates. This did not reveal any sex differences in survival or any interactions with sex (all $P > 0.10$) and we therefore dropped sex from all further analyses. Where we were explicitly interested in the timing of birth relative to other years we used standardized birth date as the number of days past December 10. Where we were interested in the timing of birth relative to the population average (to control for annual variation in average birth date) we used a measure based on setting the average birth date in the population in that year equal to zero. All data reported are as means ± SE unless otherwise stated.

Calculation of selection gradients and visualization of fitness surfaces

We used logistic regression to test for determinants of offspring survival and the method of Janzen and Stern (1998) to statistically test for significance and transform the selection gradients to selection coefficients. Both regression variables (body mass and birth date) were standardized to a mean of zero and standard deviation to one before analyses. Selection coefficients for body mass and birth date were obtained from a regression model without interactions, whereas the squared terms and interaction were obtained by including each one separately in the model (following e.g., Calsbeek and Sinervo 2007). Squared terms and their standard errors were doubled (Stinchcombe et al. 2008). The results were confirmed using canonical analysis (PROC RSREG in SAS STAT 9.2; Phillips and Arnold 1989; Blows and Brooks 2003), which generated virtually identical results for quadratic and cross-product terms and we therefore only present the results from the regressions. We visualized fitness surfaces (Schluter 1988, Schluter and Nychka 1994, Brodie et al. 1995) by calculating thin-splines from a logistic model using PROC GAM (SAS STAT 9.12) and standardized our fitness measures by dividing by the average survival of offspring for each year.

Results

THERMAL OPPORTUNITY, THE TIMING OF PARTURITION, AND OFFSPRING MASS AT BIRTH

Experimental manipulation of the opportunity for thermoregulation had a strong effect on offspring birth dates in each of the replicates (i.e., years), but the strength of the effect varied between replicates and between altitudes (Table 1; Fig. 1). Overall, the highland populations had a shorter delay in birth than the lowland populations in the low basking treatment compared to the high basking treatment (the average difference in mean birth

Table 1. Output from linear models with birth date as the dependent variable. Backward elimination of nonsignificant factors resulted in a final model (in bold) including all factors except for the three-way interaction and female snout-vent length (SVL). Error df = 434 for the final model.

Fixed effects	ndf	ddf	*F*	*P*
Thermal opportunity (TO)	**1**	**344**	**622.5**	**<0.001**
Altitude	**1**	**344**	**14.2**	**<0.001**
Replicate	3	**344**	**35.4**	**<0.001**
TO × Altitude	**1**	**344**	**12.9**	**<0.001**
TO × Replicate	**3**	**344**	**6.5**	**<0.001**
Altitude × Replicate	**3**	**344**	**20.0**	**<0.001**
TO × Altitude × Replicate	3	341	2.5	0.07
Female SVL	1	340	0.7	0.40

date between the low and high opportunity for thermoregulation ranged from 19 days to 34 days in lowlands and between 19 and 25 days in highlands; Table 1; Fig. 1). The effect of maternal thermal opportunity on offspring mass also differed across altitudes; whereas poor basking opportunity consistently reduced offspring mass in the lowland there was no effect of basking treatment on offspring mass in the highlands (Table 2; Fig. 2). Overall, offspring are heavier in highland populations but the average body mass of offspring for the two populations varied substantially across the four years (Table 2).

In our two long-term study populations, cooler years result in delayed birth at the population level (Fig. 3; correlation between mean maximum temperature during gestation [October–December in lowlands, mid October–mid January in highlands] and date of first birth: Highland: $r = -0.84$; $P = 0.005$; Lowland: $r = -0.74$; $P = 0.02$, $N = 9$; see also Cadby et al. 2010). Although mean offspring mass generally declined with date of birth within years (holding female SVL constant using partial correlation; 12 out of 18 correlations being negative: Lowland: $-0.28 < r < 0.13$; Highland: $-0.40 < r < 0.15$), this only reached statistical significance in one year for each population (Lowland: year 2003; $r = -0.28$; $P = 0.003$; Highland: year 2001; $r = -0.40$; $P < 0.001$).

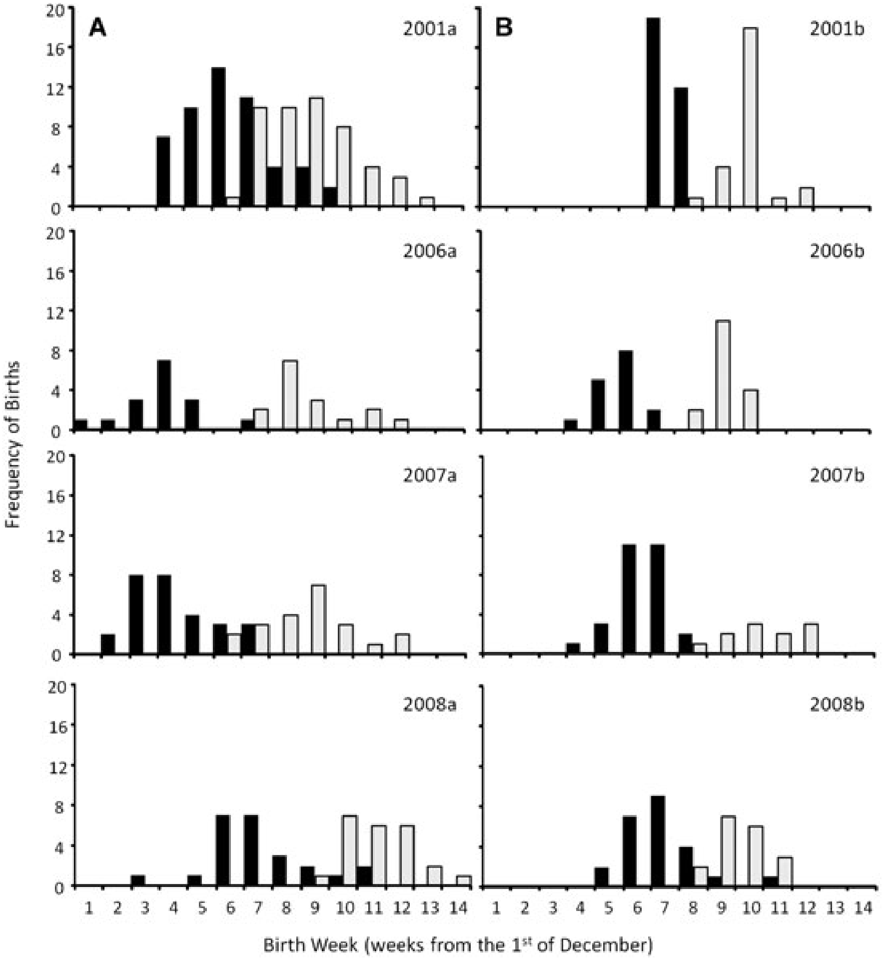

Figure 1. Frequency histograms of female birth dates for females from the lowlands (A) held under high (10 h/day) (■) and low (4 h/day) (□) basking opportunity and females from the highlands (B) held under high (■) and low (□) basking opportunity in four experimental replications carried out in 2001, 2006, 2007, and 2008.

Table 2 Output from linear mixed models with offspring mass at birth as the dependent variable. (A) Full model including both altitudes and all four replicated experiments. (B) Model for the low altitude. (C) Model for the high altitude. Backward elimination of nonsignificant factors was used and results for retained factors (in bold) refer to the final model. Degrees of freedom were estimated using the Kenward–Roger method and family was nested within the appropriate fixed effect for each model.

	(A) Both altitudes				(B) Low altitude				(C) High altitude			
Random effects	Estimate		χ^2	P	Estimate		χ^2	P	Estimate		χ^2	P
Family	2.2×10^{-3}		357.8	<0.001	2.4×10^{-3}		224.4	<0.001	2.2×10^{-3}		184.1	<0.001
Residual	1.3×10^{-3}				1.1×10^{-3}				1.4×10^{-3}			
Fixed effects	ndf	ddf	F	P	ndf	ddf	F	P	ndf	ddf	F	P
Thermal opportunity (TO)	**1**	**370**	**5.2**	**0.02**	**1**	**224**	**11.8**	**<0.001**	**1**	168	0.01	0.91
Altitude	**1**	**433**	**16.1**	**<0.001**	-	-	-	-	-	-	-	-
Replicate	**3**	**460**	**26.5**	**<0.001**	**3**	**229**	**5.2**	**0.002**	**3**	**170**	**16.0**	**<0.001**
TO × Altitude	**1**	**370**	**5.7**	**0.01**	-	-	-	-	-	-	-	-
TO × Replicate	3	470	0.8	0.48	3	225	1.2	0.32	3	167	0.1	0.98
Altitude × Replicate	**3**	**462**	**7.1**	**<0.001**	-	-	-	-	-	-	-	-
TO × Altitude × Replicate	3	447	0.7	0.56	-	-	-	-	-	-	-	-
Female SVL	**1**	**555**	**14.6**	**<0.001**	**1**	**237**	**14.6**	**<0.001**	**1**	**184**	**8.3**	**0.004**

FEMALE THERMOREGULATION

The proportion of time spent basking per day was highly repeatable within females in the laboratory in both populations and treatments (data from 2008; High basking opportunity: Lowland = 0.68; Highland = 0.52; Low basking opportunity: Lowland = 0.75; Highland = 0.50, $P < 0.01$ for all). Our laboratory data from 2006 also showed that this translated into a weaker but significant repeatability of body temperature across gestation for females from both populations and in both treatments (High basking opportunity: Lowland = 0.07; Highland = 0.09; Low basking opportunity: Lowland = 0.10; Highland = 0.15, $P < 0.05$ for all). Estimated maternal body temperatures differed both between populations ($F_{1.124} = 229.27$, $P < 0.0001$) and between thermal regimes ($F_{1.124} = 20.68$, $P = 0.0001$). Actively thermoregulating (i.e., not hiding under the shelter) females in the lowland population maintained an average estimated body temperature of 24.5 ± 0.19°C and 25.1 ± 0.23°C for the high and low thermal opportunity, respectively, whereas highland females had a body temperature of 27.3 ± 0.14°C and 28.4 ± 0.21°C for the high and low thermal opportunity, respectively. The experiment from 2008 when thermoregulation was closely monitored showed that this effect could be explained by a more active and consistent basking (i.e., increased time spent directly under the heat source) in highland females, in particular under low thermal opportunity (Altitude: $F_{1,119} = 57.47$, $P < 0.001$; Thermal Opportunity: $F_{1,119} = 45.58$, $P < 0.001$; Altitude × Thermal Opportunity: $F_{1,119} = 14.63$, $P < 0.001$; Fig. 4). Females that spent more time basking (averaged across all days for which it was measured) gave birth significantly earlier when the opportunity for thermoregulation was high (Lowland: $r = -0.70$, $P < 0.001$, $N = 25$; Highland: $r = -0.61$, $P < 0.001$, $N = 30$). The same effect was not found when the opportunity for thermoregulation was low, presumably as a result of reduced variance in thermoregulatory behavior (Lowland: $r = -0.07$, $P = 0.73$, $N = 26$; Highland: $r = -0.14$, $P = 0.54$, $N = 20$). These results held true when female SVL was controlled for in partial correlation analyses (data not shown).

SELECTION ON TIMING OF PARTURITION AND OFFSPRING MASS

There was a strong interaction effect between population and year on offspring survival ($F_{2,1641} = 12.4$, $P < 0.001$) and we therefore continued by running separate analyses for each population. This revealed that survival estimates varied across years for both populations, but there was no evidence that the effect of birth mass or birth date on survival differed significantly across years for either population (all heterogeneity of slopes tests $P > 0.25$; Table 3). Across years, body mass significantly predicted survival in the highland population, but not in the lowland population (Table 3). To generate selection coefficients, we analyzed each year

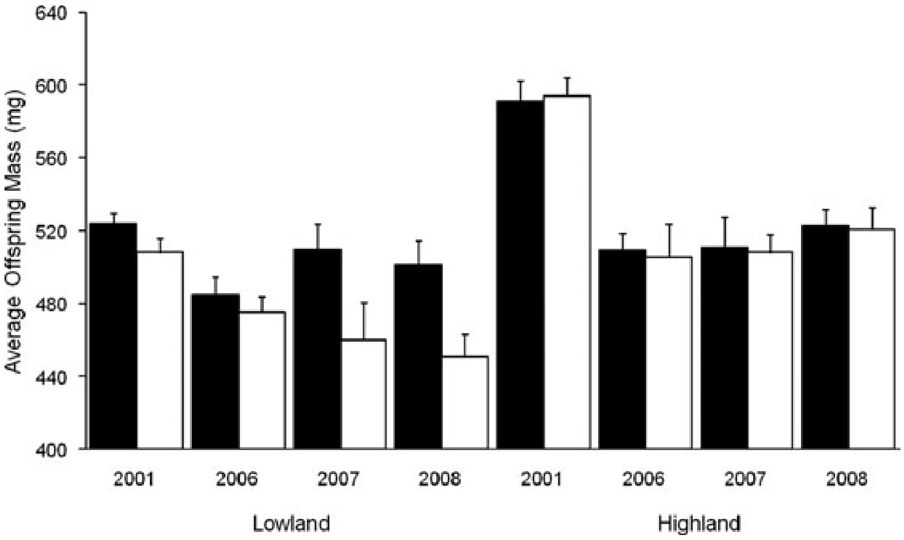

Figure 2. Difference in mean body mass (± SE) at birth for lowland and highland females held under high (10 h/day) (■) and low (4 h/day) (□) basking opportunity across four experimental replicates (2001, 2006, 2007, and 2008).

separately. There was no evidence for directional or correlational selection on body mass or birth date in any of the three years in the lowland population (Table 4; Fig. 5). In the alpine population, however, selection generally favored high body mass, in particular for later-born offspring (Table 4; Fig. 5).

REPEATABILITY AND HERITABILITY OF TIMING OF PARTURITION AND OFFSPRING MASS

Both birth date (relative to the average birth date in the population in a given year) and offspring mass were moderately repeatable within females across years in both natural populations

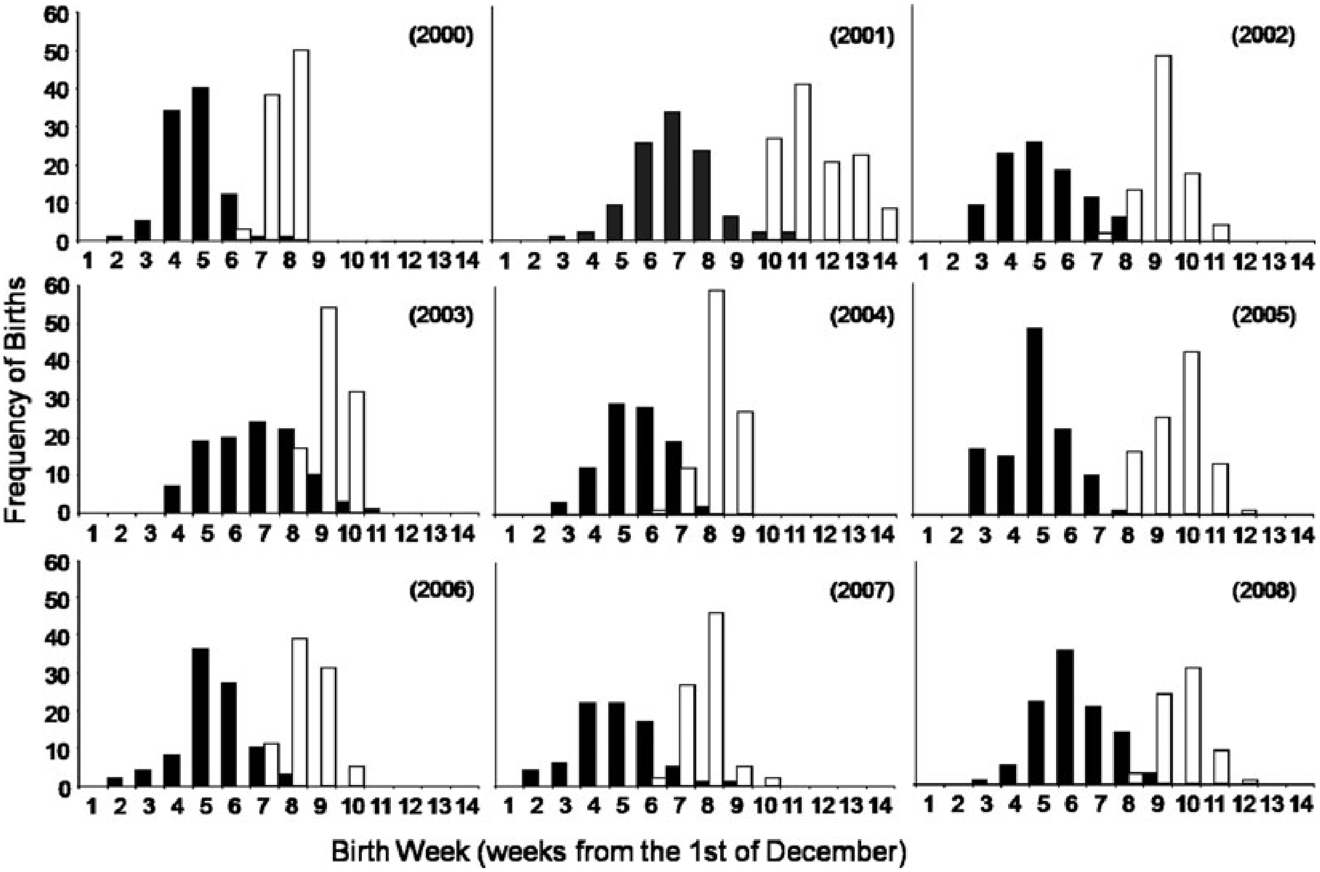

Figure 3. The number of females giving birth in the natural lowland (■) and the highland (□) populations for each of the nine years

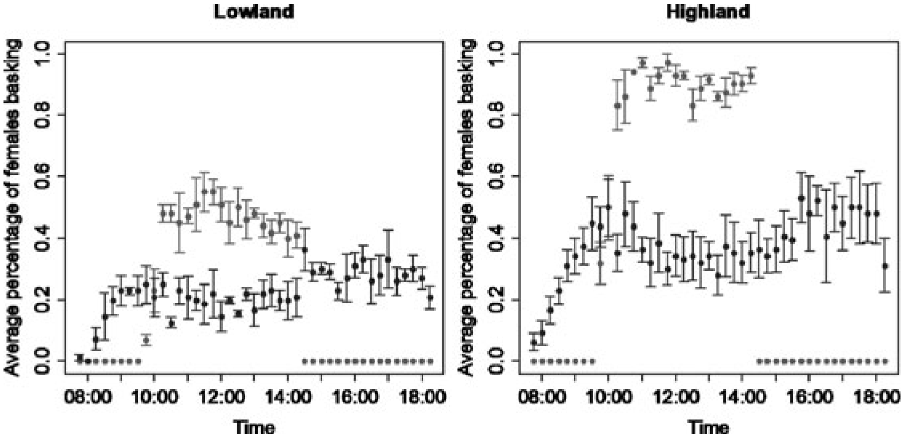

Figure 4. Average percentage of females basking under the heat source at 15-min intervals for lowland (left) and highland (right) females in high (10 h/day) and low (4 h/day) opportunity for thermoregulation. Mean and standard errors based on readings every 15 min across four days (Lowland: $N = 30$, $N = 33$; Highland: $N = 31$; $N = 29$ females for the high and low opportunity for thermoregulation, respectively).

(Birth date: Lowland = 0.47; Highland = 0.33; Body mass at birth: Lowland = 0.22; Highland = 0.32; All $P < 0.001$). However, mother–daughter regressions for each population showed that only birth date in the lowland population was heritable; the regression coefficient for birth date in the highland population was negative (Lowland: Birth date: $h^2 = 0.53 \pm 0.25$, $t = 2.12$, $P = 0.036$, $N = 83$; Body mass at birth: $h^2 = 0.02 \pm 0.30$, $t = 0.07$, $P = 0.94$, $N = 80$; Highland: Birth date: $h^2 = -0.25 \pm 0.14$, $t = -1.85$, $P = 0.067$, $N = 134$; Body mass at birth: $h^2 = 0.22 \pm 0.17$, $t = 1.26$, $P = 0.21$, $N = 128$).

Discussion

Developmental plasticity resulting from variation in the thermal environment of embryos has received substantial interest for several decades from physiologists, ecologists, and evolutionary biologists (see Deeming 2004; Angilletta 2009 for recent reviews). However, whether such effects are generators of natural selection on parental and offspring strategies, evolved adaptive plasticity, or 'passive' variation resulting from developmental processes remain unclear. Our results might clarify the evolutionary significance of effects of maternal thermal opportunity on offspring phenotype. On the basis of our results we suggest that strong effects of maternal thermal opportunity on offspring traits within populations of *N. ocellatus* are maintained because there is weak or inconsistent selection on those traits and not because they represent adaptive plasticity. Furthermore, we suggest that such passive temperature-induced phenotypic variation in offspring may contribute to evolutionary divergence of maternal behavior across climatic regimes.

Selection on offspring survival from birth through hibernation and until the beginning of the following spring favored heavier offspring at high altitude in all three years for which we had data. In contrast, there was no survival selection on mass at birth in the lowland. Our long-term studies of the two focal populations do not allow us to disentangle the causes of the differences in selection on body mass (or the relatively minor difference in correlational selection on birth date). However, it is reasonable to assume that the substantial difference in climate (including temperature) between high and low altitudes is at least partly

Table 3. Output from generalized linear mixed models with offspring survival over the first year of life as the dependent variable (binomial distribution, logit link function) for (A) a lowland and (B) a highland population. The data are based on three years (2000, 2001, 2007). Backward elimination of nonsignificant factors was used and results for retained factors (in bold) refer to the final model. All interactions between covariates and year were no-significant ($P > 0.25$ for all interactions; data now shown).

	(A) Lowland population				(B) Highland population			
Fixed effects	ndf	ddf	*F*	*P*	ndf	ddf	*F*	*P*
Year	**2**	**607**	**8.8**	**<0.001**	**2**	**1060**	**12.4**	**<0.001**
Mass at birth	1	580	0.9	0.34	**1**	**1060**	**9.2**	**0.003**
Date of birth	1	582	2.3	0.13	1	1059	0.2	0.55

Table 4. **Selection coefficients for linear (β) and nonlinear (γ) forms of natural selection on birth date and body mass at birth for juvenile *Niveoscincus ocellatus*. (A) Lowland population; (B) Highland population. Birth date and body mass were standardized to mean of zero and standard deviation of one. See text for further details on the calculation of selection coefficients. Selection coefficients with $P < 0.10$ are highlighted in bold.**

(A) Lowland population

Source for W	Year 2000				Year 2001				Year 2007			
	β/ γ	SE	χ^2	*P*	β/ γ	SE	χ2	*P*	β/ γ	SE	χ2	*P*
Birth date	0.04	0.186	0.05	0.83	−0.13	0.104	1.47	0.22	−0.09	0.106	0.75	0.39
Body mass	0.31	0.199	2.47	0.11	0.08	0.105	0.65	0.42	0.09	0.111	0.59	0.44
[Birth date]^2	0.03	0.129	0.04	0.84	0.01	0.059	0.02	0.90	0.08	0.071	1.40	0.24
[Body mass]^2	−0.02	0.167	0.01	0.92	−0.10	0.085	1.31	0.25	−0.04	0.096	0.19	0.66
Birth date × Body mass	0.16	0.185	0.71	0.40	−0.03	0.097	0.13	0.72	0.02	0.213	0.02	0.88

(B) Highland population

Source for W	Year 2000				Year 2001				Year 2007			
	β/ γ	SE	χ^2	*P*	β/ γ	SE	χ2	*P*	β/ γ	SE	χ2	*P*
Birth date	0.05	0.064	0.53	0.47	0.04	0.081	0.25	0.62	−0.10	0.107	0.85	0.36
Body mass	0.11	0.066	2.57	0.11	**0.16**	**0.088**	**3.40**	**0.06**	**0.22**	**0.106**	**4.31**	**0.04**
[Birth date]^2	−0.05	0.059	0.73	0.39	0.01	0.065	0.03	0.87	0.10	0.080	1.66	0.20
[Body mass]^2	**−0.09**	**0.052**	**3.00**	**0.08**	−0.02	0.063	0.13	0.72	0.04	0.048	0.54	0.46
Birth date × Body mass	**0.12**	**0.067**	**3.02**	**0.08**	0.07	0.084	0.64	0.43	**0.36**	**0.132**	**7.51**	**<0.01**

responsible. Hatchlings and juveniles in high-altitude populations experience cooler temperatures and are born closer to the timing of hibernation than offspring at low altitudes, which may make body size and condition at birth a more relevant predictor of survival before, during, and after hibernation (Atkins et al. 2007; Iraeta et al. 2008; see also Dobson and Michener 1995; Rieger 1996). Furthermore, population densities are similar across altitudes and years (E. Wapstra, unpubl. data), suggesting that variation in selection arising from local density (which has been shown in oviparous lizards; Svensson and Sinervo 2000; Calsbeek and Smith 2007) plays a minor role in the altitudinal pattern observed in *N. ocellatus*. However, other biotic factors may also contribute to altitudinal differences in the strength of selection, including predators and parasites.

This altitudinal divergence in selection has important implications for interpretation of the between-altitude difference in how maternal thermal opportunity affected offspring body size. Lowland females exposed to poor basking conditions gave birth to offspring with lower body mass. Phenotypic effects of average and fluctuating incubation temperature are ubiquitous in reptiles and, although the effect on the shape of hatchlings may vary between species, low temperature tend to have negative effects on size at hatching or birth (reviewed in Deeming 2004; Booth 2006). These effects are often interpreted as being adaptive because they may enable offspring phenotype to be matched to selection experienced posthatching or—parturition (Shine 1995, 2004; see also Marshall and Uller 2007; Uller 2008). Our data seem to refute this hypothesis in lowland populations of *N. ocellatus* as there was no evidence that selection favored different sized offspring at different birth dates (and thus different gestation temperatures) or in different years. Moreover, the lack of an effect of maternal thermal opportunity on offspring body mass at high altitude, where selection consistently favored large offspring size, supports the alternative hypothesis that the direct effect of maternal thermal opportunity on body mass in the lowlands may be maintained because they are selectively less important. Thus, we suggest that temperature effects on offspring development in reptiles often may be a result of weak past selection for environmental canalization rather than adaptive plasticity to match the posthatching environment. Indeed, that incubation temperature predicts selection on offspring phenotype in lizards has only been demonstrated with respect to offspring sex, which occurs because of sex differences in the benefits of early hatching or birth (Warner et al. 2009; Pen et al. 2010).

Rather than being adaptive, temperature-induced developmental plasticity with respect to body size or shape may impose strong selection on compensatory mechanisms. Physiological effects of low temperature on embryo growth may therefore

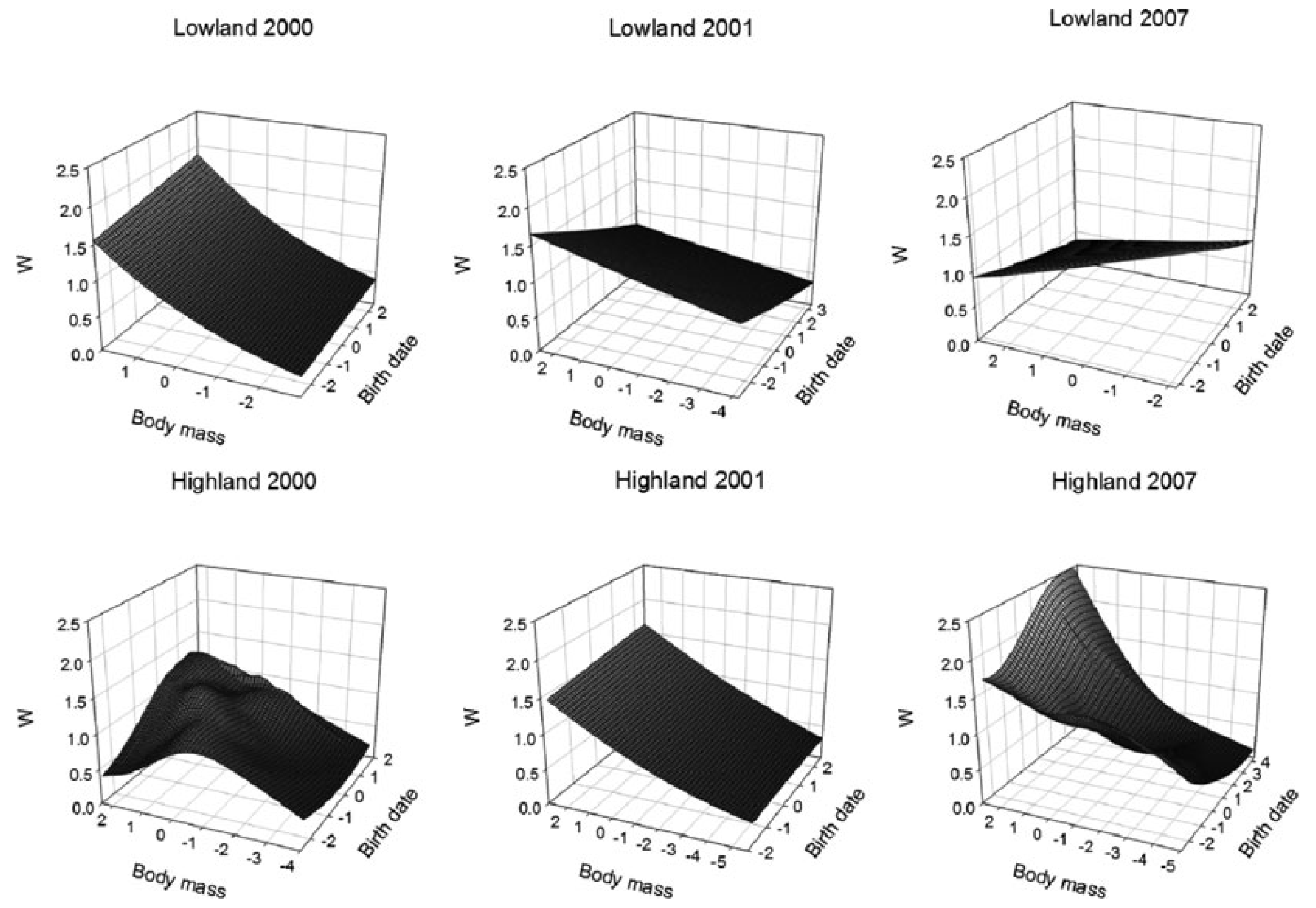

Figure 5. Selection surfaces for offspring birth date and body mass at birth for a lowland population (upper panels) and a highland population (lower panels) for three years. The populations are the same as those for which birth dates are displayed in Figure 3. See Table 4 for selection coefficients. Number of survivors/Number of released offspring (Lowland: 2000: 25/194; 2001: 65/212; 2007: 49/204; Highland: 2000: 153/369; 2001: 122/432; 2007: 76/273).

be important generators of selection on offspring and maternal morphology, physiology, and behavior (Shine 1995, 2004; see also Uller 2003; Langkilde and Shine 2005; Webb et al. 2006; Badyaev 2009). Highland female snow skinks were more active thermoregulators and maintained higher temperatures than lowland females, in particular under poor thermal conditions. Thus, highland females will tend to "buffer" their embryos from poor thermal opportunity to a greater extent than do lowland skinks, which is concordant with the observed weaker effects of thermal opportunity per se on offspring development. Furthermore, the altitudinal divergence in natural selection on offspring body mass at birth is consistent with the hypothesis that maternal behaviors may reduce the effect of thermal stress on embryo growth and development and contribute to adaptation to extreme environments (Shine 1995, 2004). However, the population divergence in the effects of maternal thermal environment on offspring body size and birth date could also be caused by a stronger canalization of maternal nutrient transfer or offspring development across different thermal environments in the highlands. These scenarios are unfortunately impossible to fully disentangle as embryos of this species cannot be cultured ex vivo. Furthermore, there are currently little empirical data on the costs and benefits of thermoregulation from the maternal perspective in this and other reptiles despite that gravid lizards frequently adjust their body temperature during gestation (reviewed in Shine 1980; Robert and Thompson 2010). Thus, we cannot infer to what extent basking trades off against other traits (e.g., predation risk; Huey and Slatkin 1976; Angilletta 2009) and explicitly test whether the altitudinal divergence in maternal behavior follow cost-benefits models of thermoregulation. Nevertheless, we suggest that passive temperature-induced phenotypic variation in offspring may be an important factor that promotes evolutionary divergence in maternal behavior when species encounter novel climatic regimes (see also Badyaev 2005, 2009).

Assessing the potential for evolutionary change in the timing of parturition and offspring body mass across climatic regimes or under climate change also requires estimates of heritability. Given the consistent selection on offspring body mass at high altitudes, we may expect genetic variation in offspring mass and birth date to be eroded to a greater extent in highland than in lowland populations. However, body mass at birth was not heritable at low or high altitude and birth date only weakly heritable in the lowland population. The high repeatability of these traits between years within natural populations may suggest that any additive genetic variance in behavioral thermoregulation is small in comparison to nongenetic variation. Furthermore, in the lowland population, consistent microvariation in the opportunity for thermoregulation may contribute to repeatability in the wild because females show high site-fidelity (G. M. While et al., unpubl data). Whether the observed differences in thermoregulatory behavior between lowland and highland females represent acclimation to thermal conditions experienced during ontogeny or genetic divergence is unknown. Regardless, within populations there seems to currently be limited potential for an evolutionary response in offspring body mass, and perhaps birth date, suggesting that future changes in breeding phenology and reproductive output under the projected temperature increase in Tasmania (Corney et al. 2010) will be dictated by plastic responses to temperature rather than genetic responses to selection.

SUMMARY

In summary, natural selection on offspring body mass was consistently positive at high altitudes, whereas selected could not be detected at low altitudes. Experimental manipulation of maternal thermal opportunity had no effect on offspring mass in highland females, whereas poor thermal opportunity reduced offspring mass in lowland females. Our data suggest that this divergence could be mediated by changes in female thermoregulatory behavior, perhaps indicating that passive developmental effects of temperature during embryonic development can be important generators of selection on maternal thermoregulatory behavior (Shine 2004, 2005; Webb et al. 2006). More generally, we suggest that phenotypic effects of maternal thermal opportunity or incubation temperature in reptiles most commonly will reflect weak selection on offspring phenotype or selection on maternal strategies rather than evolved adaptive plasticity to match postnatal environments.

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