

Evolution of viviparity in warm-climate lizards: an experimental test of the maternal manipulation hypothesis

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Abstract

The maternal manipulation hypothesis for the evolution of reptilian viviparity has been claimed to apply to any situation where gravid females are able to maintain body temperatures different from those available in external nests, but empirical data that support this hypothesis are very limited. Here, we tested this hypothesis using gravid females of a warm-climate lizard, *Mabuya multifasciata*, by subjecting them to five thermal regimes for the whole gestation period. We found gravid females selected lower body temperatures and thermoregulated more precisely than did nongravid females. Offspring produced in different treatments differed in head size, limb length and sprint speed, but not in overall body size or mass. Variation in morphological traits of offspring was induced primarily by extreme temperatures. Sprint speed of offspring was more likely affected by the mean but not by the variance of gestation temperatures. Gravid females maintained more stable body temperatures than did nongravid females not because these temperatures resulted in the optimization of offspring phenotypes but because the range of temperatures optimal for embryonic development was relatively narrow. Our data conform to the main predictions from the maternal manipulation hypothesis that females should adjust thermoregulation during pregnancy to provide optimal thermal conditions for developing embryos and that phenotypic traits forged by maternal thermoregulation should enhance offspring fitness.

Introduction

Viviparity, a reproductive mode that evolves from oviparity through gradual increases in the length of egg retention, is widespread among vertebrates. The evolutionary transition from oviparity to viviparity is of biological importance, because it likely alters the reproductive success of the organisms involved and, as such, is a major evolutionary event propelled predominantly by natural selection. Viviparity has evolved independently in more than 100 lineages of squamate reptiles (lizards, snakes and amphisbaenians), far more often than in all

other vertebrates combined (Lee & Shine, 1998; Andrews & Mathies, 2000; Blackburn, 2000; Shine, 2005). These organisms therefore provide an ideal model system to study the selective forces for the evolution of viviparity. Research on reptilian viviparity is extensive (Andrews & Mathies, 2000; Blackburn, 2000; Stewart & Thompson, 2000; Thompson *et al.*, 2002), but the ultimate causes and selective forces that led to the transition from oviparity to viviparity are still far from being well understood.

Nowadays, the widely accepted idea is that viviparity has evolved in reptiles for thermal reasons; that is, thermal differentials between the uterus and the nest resulting from maternal regulation of incubation temperature are the key to the evolution of viviparity (Blackburn, 2000; Shine, 2004, 2005; Webb *et al.*, 2006). In cold climates, for example, the negative effects

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of cold nest temperatures on developing eggs and/or the benefits of warmer incubation temperatures to eggs retained in the uterus are the primary selective forces behind the transition from oviparity to viviparity (Mell, 1929; Weekes, 1933; Sergeev, 1940; Shine, 1995). The observation that reptilian viviparity is relatively common in cold climates has given rise to the cold-climate hypothesis, which interprets a strong association between cold climates and viviparity as due to the fact that maternal thermoregulation can maintain higher body temperatures for eggs retained *in utero* than would be available in natural nests (Tinkle & Gibbons, 1977; Shine, 1995; Hodges, 2004). Nonetheless, this hypothesis cannot explain why viviparity has been such a successful strategy among reptiles in warm climates, where oviparity may be precluded not because there are no nest sites with high-enough temperatures. The results from recent studies show that not only the mean but also the variance (including its diel cycle) of temperatures experienced by developing embryos can significantly affect developmental rates and/or offspring phenotypes in reptiles (e.g. Andrews, 2000; Ji *et al.*, 2003; Shine, 2004; Webb *et al.*, 2006). These results directly or indirectly support Shine's (1995) novel hypothesis that viviparity evolves in reptiles because reproducing females can enhance offspring (and thus, their own) fitness by manipulating thermal conditions during embryogenesis.

Shine's (1995) maternal manipulation hypothesis has been claimed to apply to any situation where gravid females are able to maintain better or more predictable body temperatures for embryonic development than those available in external nests (Webb *et al.*, 2006). However, empirical data that support this hypothesis are still very limited. The hypothesis generates two main predictions that can be tested experimentally: (1) females should adjust thermoregulation during pregnancy to provide optimal thermal conditions for developing embryos; and (2) phenotypic traits forged by maternal thermoregulation should enhance offspring fitness. These predictions have been tested in a viviparous elapid snake (*Acanthophis praelongus*), where females maintaining less variable body temperatures produce larger offspring (greater body length and head size) and larger body size may enhance offspring recapture rates (indicative of survival rates) in the field (Webb *et al.*, 2006). Webb *et al.* (2006) argue that viviparity might enhance fitness in the tropics via the same pathway as in the temperate zone and that the maternal manipulation hypothesis may explain selective advantage of viviparity in tropical as well as cold-climate reptiles.

Here, we describe a study where gravid many-lined sun skinks (*Mabuya multifasciata*) were maintained under five different thermal conditions for the whole gestation period to test the maternal manipulation hypothesis. This medium-sized (to 117-mm snout-vent length, SVL; Ji *et al.*, 2006a), viviparous scincid lizard has an exclusively tropical distribution that ranges from southern China,

Myanmar, Thailand, and Indochina to India, south to Malaysia, Singapore, Indonesia, the Philippines, Indoaustralian Archipelago and New Guinea (Zhao & Adler, 1993). We used *M. multifasciata* as a model animal for two reasons. First, the skink can be easily maintained under laboratory conditions (Ji *et al.*, 2006a). Second, the skink often has no difficulty in attaining high-enough body temperatures in the field, and is therefore ideally suited to the investigation examining the applicability of Shine's (1995) maternal manipulation hypothesis for warm-climate reptiles.

Materials and methods

Collection and treatment of animals

A total of 115 adult females were collected between early March and mid-April 2005 from a previously studied population in Ledong (18°45'N, 109°10'E), Hainan, southern China. Females were brought to our laboratory in Wuzhishan (~150 km away from the collection sites), where they were individually palpated to confirm that they had just ovulated before being assigned to one of the five thermal conditions (thermal treatments). In all the five treatments between six and eight females were housed together in a 1 × 0.6 × 0.5-m³ (length × width × height) glass cage, which had a substrate consisting of 10 cm of moist soil, debris and grasses. Females were provided with mealworms (larvae of *Tenebrio molitor*), field-captured grasshoppers and water enriched with vitamins and minerals (Nekton-Rep, Nekton-product).

Females in three of the five treatments were maintained in three 3 × 4-m² controlled temperature rooms, where they could maintain their body temperatures constant at 26, 29 and 32 (±0.3)°C respectively. The fluorescent tubes in these rooms were on a 14-h light:10-h dark cycle, and the lights were automatically switched on at 05:00 hours (Beijing time) to simulate the natural light/dark cycle. Body (cloacal) temperature was taken for each female using a WMZ-3 electronic thermometer (Shanghai Medical Instrument, Shanghai, China) to verify that the mean body temperatures were controlled at the anticipated levels.

Females in the fourth treatment [hereafter the thermoregulation (TR) treatment] were maintained in a room where ambient temperatures never exceeded 20 °C. A 275-W light bulb (14-h light: 10-h dark) suspended above one end of each cage created a thermal gradient ranging from the room temperature to 60 °C (2 cm above the cage floor), and females could regulate body temperature within their voluntary range during the light phase.

Females in the fifth treatment [hereafter the nonthermoregulation (NTR) treatment] were maintained in a room where ambient temperatures, measured at 60-min intervals using a datalogger (TinyTalk TK-0014, Gemini

Table 1 The thermal environments experienced by gravid females ($n = 36$) maintained in a room without opportunities of behavioural thermoregulation (the NTR treatment).

	Mean	SE	Range
Mean temperature (°C)	26.4	0.3	23.5–31.3
Minimal temperature (°C)	14.6	0.5	12.4–20.6
Maximal temperature (°C)	38.5	0.3	36.6–42.4
Variance	30.8	0.9	15.0–44.4

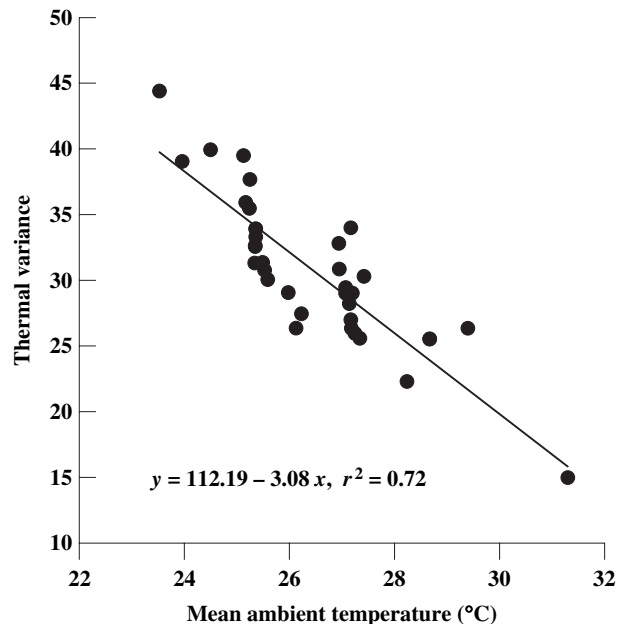


Fig. 1 The relationship between the variance and the mean of ambient temperatures experienced by gravid females in the NTR treatment. The regression equation is also indicated.

Pty, Sydney, Australia), fluctuated naturally. The room was illuminated with a natural photoperiod but shielded from direct solar radiation, and females in the treatment had no way to regulate body temperature by selective exploitation of thermal flux. Table 1 reports values for the thermal environments experienced by females in the NTR treatment. There was a negative relationship between the mean and the variance of ambient temperatures experienced by females in the treatment ($F_{1,34} = 104.47$, $P < 0.0001$; Fig. 1); body temperatures did not differ from ambient temperatures measured synchronously (paired-sample t -test; $t_{35} = 1.15$, $P = 0.259$), and ambient temperature could explain approximately 96% of variation in body temperature ($F_{1,34} = 772.78$, $P < 0.0001$).

Offspring were collected and weighed within 6 h of birth. Postpartum females were measured for SVL and tail length, weighed and then released to the site where they were collected. Females were isolated from each

other using dividers that created $30 \times 30 \times 30$ -cm³ chambers if they gave birth during the same period in the same cage, such that offspring could be allocated accurately to the mother. None of these females were isolated for more than 12 h, and a 20-W spotlight mounted in each divider isolating females in the TR treatment to allow thermoregulation. Of the 115 females, 106 gave birth to well-developed young, whereas the remaining females produced abnormal litters with various numbers of dead young, stillborns, or unfertilized eggs. Abnormal litters were found in each treatment, and were excluded from analyses.

Influence of pregnancy on mean selected body temperatures

An independent sample of 24 gravid females, 20 nongravid females and 20 adult males collected from the same population in May 2005 was used to examine the influence of pregnancy on selected body temperature (Tsel). This experiment was conducted in a $1 \times 0.6 \times 0.5$ -m³ glass cage with 10-cm depth moist soil and pieces of clay tiles. A 275-W light bulb suspended above one end of the cage created a thermal gradient ranging from 18 to 60 °C (2 cm above the cage floor) for 14 h daily. Eight to 10 skinks, with gravid females, nongravid females and adult males being randomly mixed, were moved from the cool side into the cage at 07:00 hours when the lights were switched on. Because there might be diel variation in selected body temperature, we began all measurements at 15:00 hours and ended within 2 h. Body temperatures were taken using the aforementioned thermometer. Great care was taken to avoid heat transfer occurring between the hand and the skink. To address the repeatability of our measurements, we measured each skink twice, once on each of two consecutive days. The two measures did not differ significantly (paired-sample t -test; $P > 0.25$ in all three groups), so we considered the mean of the two measures as an individual's selected body temperature.

Measurement of offspring phenotypes

Newborns were measured firstly for locomotor performance, and then for morphological traits. Because locomotor performance is thermally sensitive in reptiles, we conducted all trials at the body temperature of 30 °C, which was controlled by placing the newborns in an incubator at 30 °C for 30 min prior to testing. Locomotor performance was assessed by chasing the neonates along a 2-m racetrack with one side transparent, which allowed video-taping with a Panasonic NV-DS77 digital video camera. The racetrack was placed in a room where ambient temperatures varied from 27 to 32 °C. Each newborn was run twice with a 30-min rest between the two successive trials and, during the resting period, it was placed back to the incubator. The tapes were later

examined with a computer using MGI VideoWave III software (MGI Software Co., Toronto, Canada) for sprint speed in the fastest 25-cm interval. We cooled the newborns to approximately 8 °C, and then measured them with Mitutoyo digital calipers. The morphological measurements included SVL, tail length, head length (from the snout to the anterior edge of tympanum), head width (posterior end of the mandible), fore-limb length (humerus plus ulna) and hind-limb length (femur plus tibia).

Statistical analyses

We used Statistica software package (version 5.0 for PC) to analyse data. Data from the same litter were blocked to avoid pseudo-replication. Prior to all statistical analyses, data were tested for normality using the Kolmogorov–Smirnov test and, for homogeneity of variances, using the Bartlett's test. \log_e transformation was performed when necessary to satisfy the assumptions for using parametric tests. All values are presented as mean \pm SE, and the significance level was set at $\alpha = 0.05$.

Results

Female reproduction

Mean values for female SVL did not differ among the five treatments (ANOVA; $F_{4,101} = 1.15$, $P = 0.339$). Parturition occurred between mid-April and early July, with females at higher (average) body temperatures giving birth earlier than did those at lower body temperatures. Litter size, varying from two to seven young, did not differ among the five treatments (ANCOVA with female SVL as the covariate; $F_{4,100} = 0.87$, $P = 0.484$). Offspring mass, varying from 0.84 to 1.29 g, did not differ among the five treatments (ANCOVA with female SVL as the covariate; $F_{4,100} = 0.26$, $P = 0.904$).

Mean selected body temperatures

Mean selected body temperatures differed among gravid females, nongravid females and adult males (ANOVA; $F_{2,61} = 52.54$, $P < 0.0001$; Table 2), with gravid females selecting lower body temperatures than did nongravid females and adult males (Tukey's *post hoc* test; both $P < 0.0002$). Gravid females regulated body temperature

Table 2 Descriptive statistics of body temperatures (°C) selected by gravid females, nongravid females and adult males in the laboratory thermal gradient.

	N	Mean	SE	Range
Gravid females	24	29.0	0.2	27.6–30.8
Nongravid females	20	32.9	0.4	29.7–35.2
Adult males	20	32.5	0.3	30.2–35.7

more precisely (variance 1.14) than did nongravid females (variance 2.96) and adult males (variance 2.15). The difference in the variance of selected body temperatures was significant between gravid females and nongravid females ($F_{19,23} = 2.60$, $P < 0.05$), but not between gravid females and adult males ($F_{19,23} = 1.88$, $P > 0.05$).

Morphology of offspring

Offspring produced in different treatments differed in head length, head width, forelimb length and hind limb length, but not in SVL, tail length, or body mass (Table 3). The first two components (with eigenvalues ≥ 1) resolved by a principal component analysis (varimax rotation) from six SVL-free neonatal variables accounted for a total of 48.1% variation in the original data (Table 4). The first component (28.0% of variance explained) had higher positive loading for SVL-free values of forelimb length and hind limb length, whereas the second axis (20.1% of variance explained) had higher negative loading for SVL-free value of tail length. Offspring differed among treatments in their scores on the first axis (ANOVA; $F_{4,101} = 8.19$, $P < 0.0001$; 26^b, 29^b, 32^b, TR^b, NTR^a, Tukey's *post hoc* test, $\alpha = 0.05$, $a > b$) but not on the second axis (ANOVA; $F_{4,101} = 1.19$, $P = 0.319$) (Fig. 2). SVL-specific fore- and hind limb lengths were apparently longer in offspring produced in the NTR treatment (Table 3).

Locomotor performance of offspring

None of the examined morphometric traits was a determinant of sprint speed (linear regression analysis; all $P > 0.05$). One-way ANOVA revealed that sprint speed differed among the five treatments (\log_e -transformed data; $F_{4,103} = 16.54$, $P < 0.0001$), with offspring produced in the NTR treatment running significantly more slowly than did those produced in the other four treatments (Tukey's *post hoc* test; all $P < 0.003$; Fig. 3). Sprint speed of offspring produced in the NTR treatment was correlated positively with the mean ($F_{1,34} = 52.15$, $P < 0.0001$; Fig. 4) but negatively with the variance ($F_{1,34} = 23.34$, $P < 0.0001$; Fig. 5) of gestation temperatures. When employing a partial correlation analysis to hold the mean gestation temperature constant, we found that sprint speed of offspring produced in the NTR treatment was actually not correlated with the variance ($r = 0.09$, $t_{33} = 0.51$, $P = 0.617$) of gestation temperatures.

Discussion

Our data show that litter size and offspring size (SVL, tail length and body mass) were less sensitive to variation in gestation temperatures in *M. multifasciata*. These results are similar to those reported for *Sphenomorphus indicus* (brown forest skink), a viviparous species distributed in southern

Table 3 Size and morphology of offspring produced by females maintained under different thermal conditions.

	Thermal treatments					The results of statistical analyses
	26 °C	29 °C	32 °C	TR	NTR	
<i>n</i>	20	19	17	14	36	
Snout-vent length (mm)	33.9 ± 0.3 31.3–36.5	34.2 ± 0.4 29.4–36.0	33.8 ± 0.3 30.8–38.0	34.6 ± 0.3 31.7–35.9	34.3 ± 0.2 31.4–36.5	$F_{4,101} = 1.01, P = 0.406$
Hatchling wet mass (g)	1.03 ± 0.03 0.80–1.43	1.01 ± 0.02 0.87–1.14	0.99 ± 0.03 0.85–1.29	1.05 ± 0.03 0.87–1.24	1.05 ± 0.02 0.87–1.27	$F_{4,100} = 1.15, P = 0.340$
Tail length (mm)	43.4 ± 0.6 37.6–47.5	44.7 ± 0.8 36.2–48.8	44.5 ± 0.8 39.4–51.7	45.8 ± 0.9 39.1–49.6	44.4 ± 0.5 39.4–49.9	$F_{4,100} = 1.75, P = 0.144$
Head length (mm)	9.1 ± 0.05 8.7–9.5	9.0 ± 0.07 8.0–9.5	9.0 ± 0.06 8.6–9.6	9.1 ± 0.07 8.6–9.5	9.2 ± 0.04 8.7–9.7	$F_{4,100} = 2.78, P = 0.031$; 26 ^a , 29 ^{ab} , 32 ^{ab} , TR ^b , NTR ^a
Head width (mm)	6.4 ± 0.04 5.9–6.6	6.2 ± 0.02 5.4–6.5	6.3 ± 0.1 5.9–7.6	6.3 ± 0.05 5.9–6.5	6.1 ± 0.04 5.7–6.7	$F_{4,100} = 6.23, P < 0.0002$; 26 ^a , 29 ^{ab} , 32 ^a , TR ^{ab} , NTR ^b
Forelimb length (mm)	7.7 ± 0.1 6.5–8.6	7.7 ± 0.1 6.6–8.4	7.6 ± 0.1 6.5–8.4	7.8 ± 0.1 7.1–8.5	8.0 ± 0.1 7.1–8.9	$F_{4,100} = 6.23, P < 0.0003$; 26 ^{ab} , 29 ^b , 32 ^b , TR ^{ab} , NTR ^a
Hind limb length (mm)	9.8 ± 0.1 8.3–10.8	10.0 ± 0.1 8.9–10.7	9.7 ± 0.1 8.7–10.6	9.9 ± 0.1 9.2–10.8	10.2 ± 0.1 9.0–11.5	$F_{4,100} = 3.22, P = 0.016$; 26 ^{ab} , 29 ^{ab} , 32 ^{ab} , TR ^b , NTR ^a

Values are expressed as mean ± SE and range. SVL is the covariate in all ANCOVA models. Mean values with different superscripts differ significantly (Tukey's *post hoc* test, $\alpha = 0.05$, $a > b$). TR = females maintained in the laboratory thermal gradient; NTR = female maintained in a room without opportunities of behavioural thermoregulation.

Table 4 Loading of the first two axes of a principal component analysis on six variables of neonate traits.

	Factor loading	
	PC1	PC2
Neonate wet mass	0.413	0.514
Tail length	0.046	-0.795*
Head length	0.462	-0.449
Head width	-0.292	-0.314
Forelimb length	0.800*	-0.100
Hind limb length	0.755*	0.027
Proportion of variance explained (%)	28.0	20.1

Size effects are removed in all cases by using residuals from the regressions on snout-vent length of neonates. All data were \log_e transformed.

*Variables with the main contribution to each factor.

China, India, Indochina and Malay Peninsula (Ji *et al.*, 2006b). As in *S. indicus*, other offspring phenotypes, such as head size, limb length and sprint speed, are significantly affected by gestation temperature in *M. multifasciata*. Thus, *M. multifasciata* is amongst reptilian species where thermal conditions experienced during embryogenesis can affect morphological, physiological and/or behavioural phenotypes of offspring (e.g. Deeming, 2004). In the following, we discuss our results in the framework of Shine's (1995) maternal manipulation hypothesis.

Females shift selected body temperatures when they are gravid

Selected body temperature represents the set point of thermoregulation at which numerous physiological and

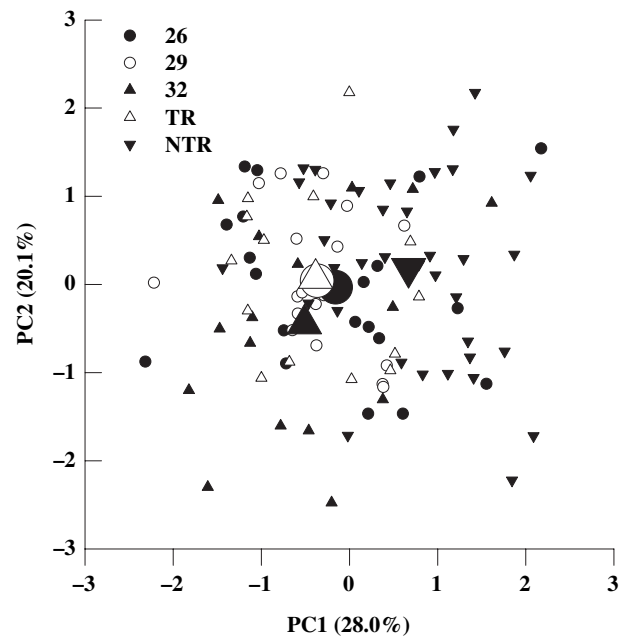


Fig. 2 Positions of offspring produced by females maintained under different thermal conditions in the space defined by the first two axes of a principal component analysis based on six size-adjusted morphological variables of offspring. Enlarged symbols show the mean values for scores on the two axes. 26, 26 and 32: females whose body temperatures were maintained at 26, 26 and 32 °C respectively; TR: females maintained in the laboratory thermal gradient; NTR: female maintained in a room without opportunities of behavioural thermoregulation.

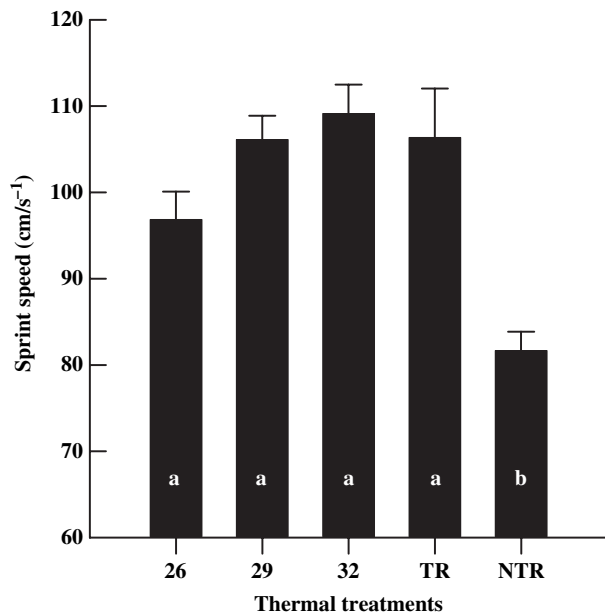


Fig. 3 Mean values (+SE) for sprint speed of offspring produced by females maintained under different thermal conditions. Means with different letters differ significantly (Tukey's *post hoc* test, $\alpha = 0.05$; a > b)

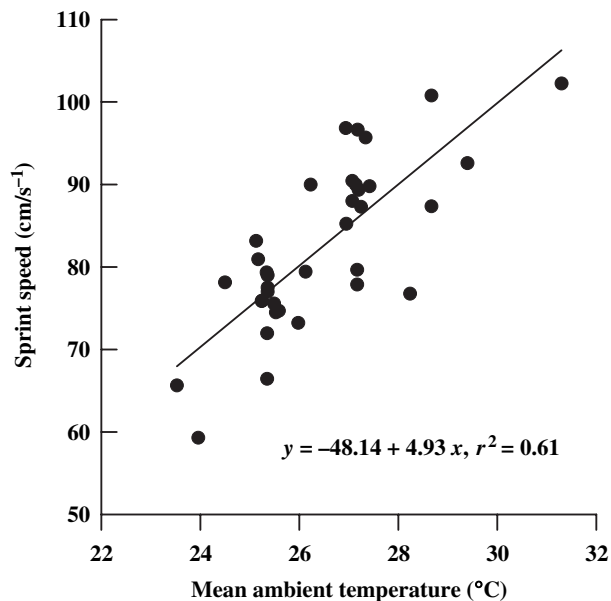
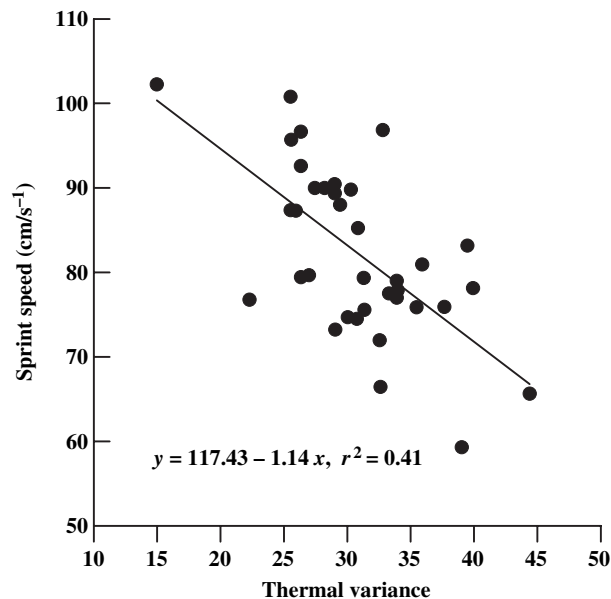


Fig. 4 The relationship between sprint speed of offspring and the mean of ambient temperatures experienced by females in the NTR treatment. The regression equation is also indicated. Body temperatures did not differ from ambient temperatures measured synchronously (see text for details).

behavioural processes function at some high levels (Hertz *et al.*, 1993; Christian & Weavers, 1996; Angilletta *et al.*, 2002); Tsel is not a fixed trait of a species, because it may



the effects of adverse biotic and abiotic factors in their general environment and/or the increased energy demands for carrying developing embryos. In this study, embryos could develop successfully at constant temperatures ranging from 26 to 32 °C without important modifications to offspring phenotypes (Figs 2 and 3). This suggests that gestation temperatures within this range almost exert no differential effects on offspring phenotypes. However, as the mean gestation length decreases much less dramatically from 29 to 32 °C (<5 days) than from 26 to 29 °C (~3 weeks) in the species (Ji *et al.*, 2006a), we expect that gravid females would benefit very little from selecting body temperatures higher than 29 °C in terms of the reduced gestation length.

Body temperatures within the range of 30–36 °C maximize swimming stamina (indicative of physiological performance) in *M. multifasciata* (X. Ji, C.-X. Lin, Y. Du *et al.*, unpubl. data). This partly explains why nongravid females and adult males select body temperatures generally falling within this range (Table 2). Feeding and swimming performances are, however, much worse in gravid females than in nongravid females and adult males, primarily because the body volume of females is highly packed when they are gravid (X. Ji, C.-X. Lin, Y. Du *et al.*, unpubl. data). Thus, shifting selected body temperatures to a lower level might be a strategy adopted by gravid females to reduce energetic costs (and thus, reproductive costs) associated with the increased metabolic rates at high body temperatures.

Offspring produced at 29 °C and in the TR treatment were almost the same with respect to the thermally plastic traits examined in this study (Figs 2 and 3). The match of offspring phenotypes between these two treatments is unlikely to come upon by chance but provides further evidence that, as in other species of lizards (e.g. Beuchat, 1988; Braña, 1993; Mathies & Andrews, 1997; Ji *et al.*, 2006b), body temperatures selected by gravid females are optimal for embryonic development in *M. multifasciata*.

Stable gestation temperatures do not optimize morphological traits of offspring

Reptiles regulate body temperatures mainly by behavioural mechanisms, such as habitat selection, restriction of periods of activity and selective exploitation of short-term environmental variations of thermal flux (Bartholomew, 1982). For reptiles living in the environments lacking temporally and/or spatially variable heat sources, variation in body temperature often mirrors variation in ambient temperature because behavioural thermoregulation can be rather constrained in these environments (Ji *et al.*, 2002a; Sun *et al.*, 2002; Pan *et al.*, 2003; Zhang *et al.*, 2003). This was also true of females in the NTR treatment, as their body temperatures varied almost coincidentally with ambient temperatures encountered.

The thermal regimes that females encountered in the NTR treatment varied seasonally, with ambient temperatures (and thus, gestation temperatures) being lower and more variable early than later in the season. Offspring produced at lower and more variable gestation temperatures ran more slowly than did those produced at warmer and more stable temperatures (Figs 4 and 5), seemingly signifying that both the mean and the variance of gestation temperatures affected sprint speed of offspring. Interestingly, however, sprint speed was actually not correlated with the thermal variance when holding the mean temperature constant. This result is similar to that reported for *Eumeces chinensis* (Chinese skink; Chen *et al.*, 2003), but differs from that reported for *Takydromus septentrionalis* (northern grass lizard) where hatchlings from unstable incubation temperatures perform better in the racetrack than do those from stable incubation temperatures (Du & Ji, 2006).

Temperatures over some upper thresholds can substantially affect morphological phenotypes of offspring in lizards (e.g. Braña & Ji, 2000; Ji *et al.*, 2002b, 2006b; Du & Ji, 2006). Thus, avoidance of even brief exposure of developing embryos to extreme temperatures could have been the selective basis for the evolution of viviparity in reptiles (Beuchat, 1988; Mathies & Andrews, 1997). Offspring produced in the NTR treatment were morphologically different from those produced in the other four treatments (Fig. 2), primarily because they had longer SVL-specific fore- and hind limb lengths (Table 3). These differences were presumably induced by extremely high temperatures (up to 36.6–42.4 °C) ever experienced by gravid females in the treatment.

Our study is the first to demonstrate that the mean rather than the variance of gestation temperatures is more likely to affect locomotor and morphological phenotypes of offspring in viviparous reptiles. Gravid females of *M. multifasciata* regulate body temperature more precisely and, thus, maintain more stable body temperatures than do nongravid females, not because stable maternal temperatures result in the optimization of offspring phenotypes but because the range of temperatures optimal for embryonic development is relatively narrow in the species.

Phenotypic traits forged by maternal thermoregulation enhance offspring fitness

Body temperatures selected by gravid females of *M. multifasciata* varied within the range of 27.6–30.8 °C. Offspring produced in the TR treatment were quite similar to those produced at temperatures of 26–32 °C. Despite the implications of this phenotypic consistency for offspring fitness in field conditions remaining unexplored, our data also provide a support for the prediction that phenotypic traits forged by maternal thermoregulation should enhance offspring fitness, because variation in offspring phenotypes is often a major predictor of

fitness in reptiles (e.g. Webb & Cooper-Preston, 1989; Downes & Shine, 1999; Braña & Ji, 2000; Du & Ji, 2006). In fact, modification of thermoregulatory behaviour and thermal preference of gravid females could have evolved because of the advantages associated with the thermal optimality that gravid females provide to embryos, and this has been proposed as one plausible cause for the evolution of reptilian viviparity (Shine, 1995).

Conclusions

Our data conform to the predictions from the maternal manipulation hypothesis that females should shift selected body temperatures during pregnancy to provide optimal thermal conditions for developing embryos and that phenotypic traits forged by maternal thermoregulation should enhance offspring fitness. Our data do not validate the prediction that stable maternal temperatures should favour the evolution of viviparity (Webb *et al.*, 2006), because the mean rather than the variance of gestation temperatures is a more likely factor affecting offspring phenotypes in *M. multifasciata*.

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