Evol Ecol (2009) 23:777–790
DOI 10.1007/s10682-008-9272-2

ORIGINAL PAPER

Evolution of viviparity in cold-climate lizards: testing the maternal manipulation hypothesis

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Received: 10 March 2008 / Accepted: 23 June 2008 / Published online: 2 July 2008

Abstract We used a cold-climate viviparous lacertid lizard (*Eremias prezwalskii*) as a model system to test the maternal manipulation hypothesis. Twenty-four gravid females collected from a population in Inner Mongolia (northern China) were maintained in the laboratory for the whole gestation length, with 12 allowed to bask for 14-h daily and the other 12 for 10-h daily. Females selected lower body temperatures but did not thermoregulate more precisely when gravid. The mean gestation length was shorter in females provided with longer basking opportunity. Neonates in the two treatments differed in tail length and the number of ventral scales but not in other examined morphological traits, with offspring born in the 14-h treatment having longer tails but fewer ventral scales. Offspring were sexually dimorphic at birth, with females being smaller in tail length, head length and fore-limb length but having more ventral scales than males of the same size. Offspring born in the 14-h treatment were not only faster runners but also grew faster than did offspring born in the 10-h treatment. Our data validate the main predictions of the maternal manipulation hypothesis that females should shift selected body temperatures during gestation to provide optimal thermal conditions for developing embryos and that phenotypic traits determined by maternal thermoregulation should enhance offspring fitness. Our study is the first to demonstrate that the maternal manipulation hypothesis applies to cold-climate viviparous reptiles.

Keywords *Eremias prezwalskii* · Lacertidae · Maternal basking · Viviparity · Morphology · Locomotor performance · Growth · Cold-climate

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Introduction

Viviparity, a reproductive mode that evolves from oviparity via progressive increases in the duration of uterine retention of developing embryos, has had at least 150 independent origins within fishes (Goodwin et al. 2002; Reynolds et al. 2002), amphibians (Duellman and Trueb 1986) and reptiles (Shine 1985; Blackburn 1982, 1985, 1999, 2000). Viviparity has evolved independently in more than 100 lineages of squamate reptiles (lizards, snakes and amphisbaenians), far more than in all other non-mammalian vertebrates combined (Shine 2005). Squamate reptiles therefore provide excellent model systems in which to study the selective forces for the transition from oviparous to viviparous reproduction, and the adaptive significance of this transition. Many hypotheses have been proposed since the first half of the last century on the selective forces that led to the evolution of viviparity within squamate reptiles. Of these hypotheses, the cold-climate hypothesis (Tinkle and Gibbons 1977; Qualls 1997; Qualls and Shine 1998; Andrews 2000; Hodges 2004), the predictability hypothesis (Tinkle and Gibbons 1977; Shine 2002) and the maternal manipulation hypothesis (Shine 1995, 2004; Webb et al. 2006) are most frequently cited. The three hypotheses agree on the idea that viviparity has evolved in squamate reptiles for thermal reasons; that is, thermal differentials between the uterus and the nest resulting from maternal thermoregulation are the key to the evolution of viviparity (Mell 1929; Weekes 1933; Sergeev 1940; Shine 1995, 2004, 2005; Blackburn 2000).

The cold-climate hypothesis purports that viviparity evolves in cold climates because females that retain their developing embryos *in utero* will have greater reproductive success than females that lay their eggs in a nest. The predictability hypothesis, in contrast, proposes that the environmental variability of the cold or temperate regions provides selective pressures responsible for egg-retention and for the evolution of viviparity. While these two hypotheses are supported by empirical data collected in some reptiles (Shine 1983, 1987, 2002; Qualls and Shine 1998; Andrews 2000; Hodges 2004), they both cannot answer the question of why viviparity has been such a successful strategy among reptiles in warm or predictable climates. The maternal manipulation hypothesis has received increasing scientific attention over the past few years, because it seems to apply to any situations or species where gravid females are able to enhance offspring (and thus, their own) fitness by maintaining more stable body temperatures for developing embryos than those available in external nests (Shine 1995, 2004; Webb et al. 2006).

The maternal manipulation hypothesis generates two main testable predictions: (1) females should adjust thermoregulation when gravid such that they provide optimal thermal conditions for developing embryos; and (2) phenotypic traits determined by maternal thermoregulation should enhance offspring fitness. These predictions have received little empirical scrutiny, yet recent studies of two warm-climate viviparous reptiles, *Acanthophis praelongus* (northern death adder; Webb et al. 2006) and *Mabuya multifasciata* (many-lined sun skink; Ji et al. 2007), support them to be true. Might viviparity enhance fitness in cold-climate regions via the same pathway as in the tropics? Might the maternal manipulation hypothesis explain selective advantages of viviparity in cold-climate as well as tropical reptiles? To answer these questions, one urgently needs data on viviparous reptiles in cold or unpredictable climates.

Here, we describe a study where gravid Gobi racerunners (*Eremias prezwalskii*) were provided with two different basking opportunities (see below for details) for the whole gestation length to test the maternal manipulation hypothesis. This medium sized (to 95-mm snout-vent length, SVL; Zhao 1999), viviparous lacertid lizard has an exclusive temperate distribution that ranges from the northern and northwestern parts (Gansu, Inner Mongolia, Ningxia, Qinghai and Xinjiang) of China to Mongolia and Russia (southern Tuvin District) (Zhao and Adler 1993). The areas occupied by the lizard are characterized by a low mean but great amplitude of thermal fluctuations that may be an important factor restricting basking opportunities for gravid females and the thermal conditions experienced by developing embryos. Therefore, *E. prezwalskii* is ideally suited to examining the applicability of the maternal manipulation hypothesis for cold-climate reptiles.

Materials and methods

Collection and treatment of reproducing females

Twenty-four adult females (>75-mm SVL) with large-sized yolking follicles and copulation marks were collected by hand or noose between 12 and 19 May 2007 from a population in Wulatehouqi (41 45′ N, 107 01′ E; ~1,550-m elevation), Inner Mongolia (northern China). Females were placed singly in cloth bags and transported to our laboratory at Hangzhou Normal University, where they were housed in a 2.5 × 1.2 × 0.6-m^3 (length × width × height) glass cage, in an indoor animal holding facility. The cage contained a substrate of sand, with rocks and pieces of clay tiles provided as cover. During daylight hours, thermoregulatory opportunities were provided by a 200-W incandescent lamp suspended over one end of the cage; overnight temperatures followed indoor ambient temperatures (18 ± 1°C). Food (mealworms (larvae of *Tenebrio molitor*), house crickets (*Achetus domesticus*) and field-captured grasshoppers) and fresh water were provided daily, so that excess food was always available in the cage. Females were individually palpated every other day to confirm that they had just ovulated before being assigned to one of the two experimental treatments (basking opportunities). None of these females ovulated later than late May. Twelve females were allowed to bask for 14-h daily (hereafter the LT (longer thermoregulation) treatment), and the remaining 12 females for 10-h daily (hereafter the ST (shorter thermoregulation) treatment). The LT and ST treatments were designed to simulate the maximal basking opportunity available to lizards in the breeding season's sunny and cloudy days, respectively. This experimental design allow us to test the prediction that reaction norms for plastic traits may be such that females provided with limited basking opportunity would produce offspring phenotypes with particular attributes that are associated with low fitness.

Females in the LT and ST treatments were housed separately in two 1.2 × 0.7 × 0.5-m^3 plastic cages, which had a substrate consisting of 10-cm of sand and pieces of clay tiles. Females were provided with mealworms and house crickets and water enriched with vitamins and minerals. The two cages were placed in a 4 × 3-m^2 AAPS (artificial atmospheric phenomena simulator) room where the temperature was controlled at 18.0 ± 1.0°C and the fluorescent tubes, which were automatically switched on at 07:00-h (Beijing time), were on a cycle of 14-h light: 10-h dark. A 200-W incandescent lamp suspended above one end of each cage created a thermal gradient ranging from the room temperature to 55°C (2-cm above the cage floor), so that females could regulate body temperatures within their voluntary range during the light phase and cool to the room temperature when the heating light was switched off.

The cages were checked twice daily for newborns as soon as the first female gave birth, such that newborns could be always collected and weighed within a few hours after parturition. Body mass, SVL and tail length were taken for each postpartum female. Females were isolated from each other using dividers that created 30 × 30 × 30-cm^3

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 was isolated for more than 24 h, and a 20-W spotlight mounted in each divider isolating females to allow thermoregulation. Of the 24 females, 21 gave birth to well-developed young, and the remaining three produced abnormal litters with various numbers of dead young, or stillborns. Abnormal litters, one in the LT treatment and two in the ST treatment, were excluded from analyses. We calculated relative litter mass by dividing litter mass by the postpartum female mass, and relative fecundity by using the residuals derived from the regression of $\log_e$ (litter size) on $\log_e$ (maternal SVL) (Olsson and Shine 1997).

Influence of pregnancy on mean selected body temperatures

An independent sample of 10 gravid females, 10 non-gravid females and 10 adult males ($>$ 75-mm SVL) collected from the same population in mid-July 2007 was used to examine whether females shift their thermal preferences when gravid. The experiment was conducted in a plastic cage as described above. A 200-W incandescent lamp suspended above one end of the cage created a thermal gradient ranging from 18 to 55°C for 14 h daily. Ten lizards, with gravid females, non-gravid females and adult males being randomly mixed, were introduced into the thermal gradient at the cool side at 07:00-h when the lights were switched on. To remove the possible influence of diel variation in selected body temperature, we began all measurements at 15:00-h and ended within 2 h. Body (cloacal) temperature (to the nearest 0.01°C) was taken for each lizard using a UT325 digital thermometer (Shanghai Medical Instruments, China). We measured each lizard three times, once on each of three consecutive days. The three measures did not differ significantly (repeated measures ANOVA; $P > 0.218$ in all three groups), so we considered the mean of the three measures as a lizard’s selected body temperature.

Measurement of offspring phenotypes

Offspring ($N = 75$), numbered individually, often at 10-day intervals, using a non-toxic waterproof marker for future identification, were measured at birth firstly for locomotor performance and then for morphological traits. We conducted all running trials at the body temperature of 30°C, which was achieved 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-long racetrack with one side transparent, which allowed videoing with a Panasonic NV-DS77 digital video camera. The racetrack was kept in a room set at a constant 30°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 in the incubator. The tapes were later examined with a computer using MGI VideoWave III software for PC (MGI Software Co., Toronto, Canada) for sprint speed in the fastest 25-cm interval and the maximal length (the maximal length traveled without stopping).

Immediately following running trials, we cooled newborns to about 5°C, and then measured them (to the nearest 0.01 mm) with Mitutoyo digital calipers. Morphological measurements taken for each newborn included: SVL, tail length, abdomen length (from the posterior base of the fore-limb to the anterior base of the hind-limb), head length (from the snout to the anterior edge of the tympanum), head width (taken at the posterior end of the mandible), fore-limb length (humerus plus ulna), hind-limb length (femur plus tibia) and the number of ventral scales. Newborns were sexed by gently pressing on both sides of tail base using forceps to check for the presence or absence of hemipenes; the presence of hemipenes allowed unequivocal sex assignation of males.

Following morphological measurements, we randomly moved newborns into one of the six $0.5 \times 0.4 \times 0.4\text{-cm}^3$ plastic cages placed in a room set at a constant 20°C. A 60-W spotlight was mounted in each cage to allow thermoregulation for 12 h daily. Small mealworms and house crickets were provided in excess and spread throughout the cage, such that newborns had free access to the food. We evaluated early growth (the first 120-day period) by weighing offspring at 15-day intervals.

Statistical analyses

Data were analyzed using Statistica software package (Version 5.0 for PC). Data on offspring of the same sex were blocked by the litter to avoid pseudo-replication. All 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 ± SE, and the significance level is set at $\alpha = 0.05$.

Results

Female reproduction

Females produced a single litter of 2–5 young per breeding season (Table 1). Females gave birth between 7 and 30 July in the LT treatment, and between 24 July and 17 August in the ST treatment; the mean gestation length was shorter in females provided with longer basking opportunity (Table 1). Mean values for female SVL, postpartum body mass, litter

Table 1 Descriptive statistics for female reproductive traits of *Eremias prezwalskii*

Variable	Treatments		The results of statistical analyses
	Basking for 14-h daily	Basking for 10-h daily	
N	11	10	
Snout-vent length (mm)	80.2 ± 1.4 (75.4 − 87.0)	80.4 ± 1.3 (75.4 − 87.3)	$F_{1,\,19} = 0.01, P = 0.915$
Postpartum body mass (g)	10.2 ± 0.6 (7.1 − 13.6)	10.0 ± 0.4 (8.6 − 12.1)	$F_{1,\,18} = 0.26, P = 0.619$
Gestation length (days)	59.0 ± 1.5 (51.0 − 68.0)	71.0 ± 1.0 (66.0 − 75.0)	$F_{1,\,19} = 41.66, P < 0.0001$
Litter size	3.8 ± 0.4 (2 − 5)	3.3 ± 0.3 (2 − 4)	$F_{1,\,18} = 3.27, P = 0.087$
Litter mass (g)	2.61 ± 0.26 (1.54 − 4.00)	2.42 ± 0.22 (1.43 − 3.26)	$F_{1,\,18} = 2.39, P = 0.139$
Neonate mass (g)	0.71 ± 0.03 (0.53 − 0.82)	0.74 ± 0.05 (0.52 − 1.09)	$F_{1,\,19} = 0.43, P = 0.521$
Relative litter mass	0.26 ± 0.02 (0.12 − 0.38)	0.24 ± 0.02 (0.15 − 0.33)	$F_{1,\,18} = 1.06, P = 0.317$

Values are expressed as mean ± SE (range). *F* values of ANOVAs (for SVL, gestation length and neonate mass) or ANCOVAs (for postpartum body mass, litter size and litter mass with SVL as the covariate, and for relative litter mass with postpartum body mass as the covariate)

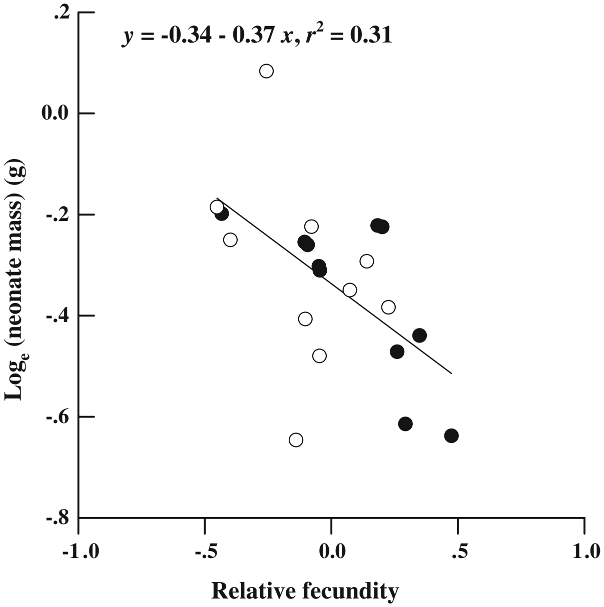

Fig. 1 The trade-off between offspring size and relative fecundity (see text for the definition). All data are log$_e$ transformed, and the regression equation is indicated in the figure. Solid dots represent neonates born in the LT (14-h basking) treatment, and open dots represent neonates born in the ST (10-h basking) treatment

size, litter mass, neonate mass and relative litter mass did not differ between the two treatments (Table 1). Neither in the LT treatment ($F_{1,\ 9} = 4.04$, $P = 0.075$) nor in the ST treatment ($F_{1,\ 8} = 0.26$, $P = 0.625$) was neonate mass dependent on maternal SVL. Data pooled for the two treatments showed once again that neonate mass was independent of maternal SVL ($F_{1,\ 19} = 2.85$, $P = 0.108$). Neonate mass showed a significant negative correlation with relative fecundity ($r = -0.56$, $F_{1,\ 19} = 8.54$, $P < 0.009$), suggesting that the trade-off between size and number of offspring was evident in *E. prezwalskii* (Fig. 1).

Selected body temperature

Gravid females, non-gravid females and adult males differed from each other in thermal preference (ANOVA; $F_{2,\ 27} = 60.68$, $P < 0.0001$; Table 2). The mean selected body temperature was lowest in gravid females and highest in adult males, with non-gravid females in between (Tukey's post hoc test; $P < 0.0002$ in all paired comparisons). Gravid females (variance = 1.20), non-gravid females (variance = 0.42) and adult males (variance = 0.47) did not differ from each other in the variance of selected body temperatures (two-tailed variance ratio test; $P > 0.05$ in all paired comparisons).

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

	N	Mean	SE	Range
Gravid females	10	30.4^c	0.3	29.1–32.4
Non-gravid females	10	32.5^b	0.2	31.6–33.3
Adult males	10	34.5^a	0.2	33.1–35.5

Means with different superscripts differ significantly (Tukey's post hoc test, $\alpha = 0.05$; a > b > c)

Offspring phenotype

None of the examined morphometric traits was a determinant of the two locomotor variables (the maximal length and sprint speed), and sex was not a significant source of variation in these two variables ($P > 0.05$ in all cases). We therefore used one-way ANOVA with basking opportunity as the factor to analyze data pooled for sexes. This analysis showed that offspring born in the two treatments differed significantly in sprint speed ($F_{1,\,19} = 9.57$, $P < 0.006$) but not in the maximal length ($F_{1,\,19} = 2.91$, $P = 0.104$), with offspring born in the LT treatment being faster runners (Fig. 2).

Neonates in the two treatments differed in tail length and the number of ventral scales, but not in other examined morphological traits (Table 3). When removing the influence of variation in SVL, we found that newborns had longer tails but fewer ventral scales in the LT treatment than in the ST treatment (Table 3). Offspring were sexually dimorphic at birth, with females being smaller in tail length, head length and fore-limb length but having more ventral scales than males of the same SVL (Table 3).

Growth in the first 120-day period was evident in both treatments (repeated measures ANOVA; LT-$F_{8,\,80} = 141.33$, ST-$F_{8,\,64} = 333.64$, $P < 0.0001$ in both cases). Offspring

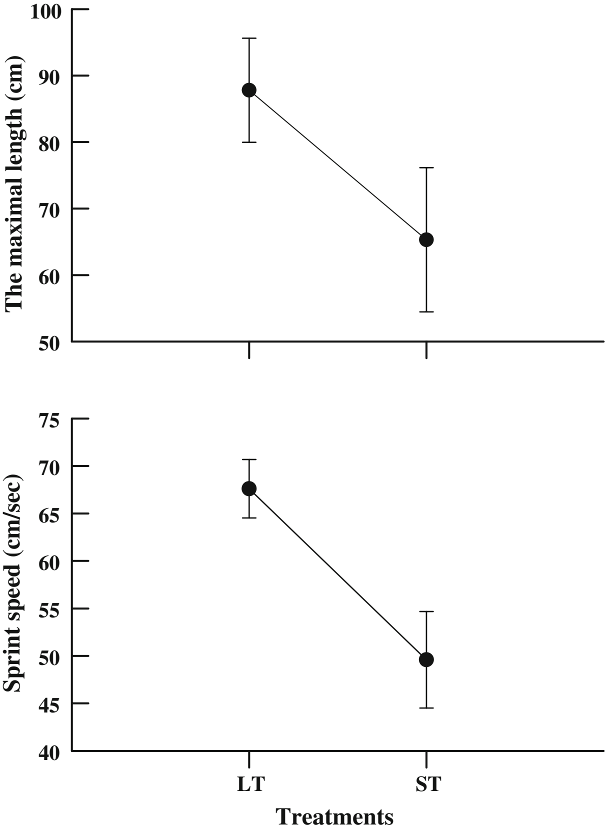

Fig. 2 Mean values (±SE) for locomotor performance (the maximal length and sprint speed) of neonates born in the LT and ST treatments. Offspring born in the two treatments differed significantly in sprint speed ($F_{1,\,19} = 9.57$, $P < 0.006$) but not in the maximal length ($F_{1,\,19} = 2.91$, $P = 0.104$)

Table 3 Size and morphology of offspring produced by females provided with two different basking opportunities

Variables	Basking for 14-h daily		Basking for 10-h daily		The results of statistical analyses		
	Females	Males	Females	Males	Sex	Treatment	S × T
N	8	7	8	9			
Snout-vent length (mm)	29.2 ± 0.6	29.3 ± 0.6	29.5 ± 0.8	29.0 ± 0.6	$F_{1,\,28} = 0.13$, $P = 0.725$	$F_{1,\,28} = 0.005$, $P = 0.942$	$F_{1,\,28} = 0.17$, $P = 0.679$
	27.1 – 32.1	26.8 – 31.3	26.0 – 33.0	25.0 – 30.8			
Tail length (mm)	37.0 ± 1.8	39.7 ± 1.9	36.6 ± 1.8	36.7 ± 1.4	$F_{1,\,27} = 5.74$, $P = 0.024$; F < M	$F_{1,\,27} = 5.13$, $P = 0.031$; LT > ST	$F_{1,\,27} = 0.68$, $P = 0.416$
	31.6 – 44.7	29.4 – 44.7	27.7 – 44.7	30.3 – 41.2			
Abdomen length (mm)	13.0 ± 0.3	12.9 ± 0.3	13.2 ± 0.5	13.1 ± 0.4	$F_{1,\,27} = 0.007$, $P = 0.933$	$F_{1,\,27} = 0.73$, $P = 0.400$	$F_{1,\,27} = 1.20$, $P = 0.283$
	11.8 – 14.4	11.8 – 13.7	11.0 – 14.9	10.7 – 14.6			
Head length (mm)	7.2 ± 0.1	7.4 ± 0.2	7.4 ± 0.1	7.5 ± 0.1	$F_{1,\,27} = 11.12$, $P < 0.003$; F < M	$F_{1,\,27} = 4.06$, $P = 0.054$	$F_{1,\,27} = 0.05$, $P = 0.823$
	6.6 – 7.7	6.5 – 7.8	6.8 – 8.0	7.0 – 7.8			
Head width (mm)	5.0 ± 0.06	5.1 ± 0.04	5.1 ± 0.07	5.1 ± 0.02	$F_{1,\,27} = 0.31$, $P = 0.583$	$F_{1,\,27} = 1.98$, $P = 0.171$	$F_{1,\,27} = 0.11$, $P = 0.742$
	4.9 – 5.4	4.9 – 5.2	4.9 – 5.5	5.0 – 5.2			
Fore-limb length (mm)	6.2 ± 0.3	6.6 ± 0.3	6.1 ± 0.3	6.2 ± 0.2	$F_{1,\,27} = 5.04$, $P = 0.033$; F < M	$F_{1,\,27} = 2.96$, $P = 0.097$	$F_{1,\,27} = 0.47$, $P = 0.478$
	5.2 – 7.3	4.8 – 7.3	4.7 – 7.6	4.8 – 6.7			
Hind-limb length (mm)	8.4 ± 0.4	8.9 ± 0.4	8.7 ± 0.5	8.6 ± 0.4	$F_{1,\,27} = 3.37$, $P = 0.078$	$F_{1,\,27} = 0.02$, $P = 0.900$	$F_{1,\,27} = 0.24$, $P = 0.629$
	7.1 – 10.4	6.9 – 9.9	6.8 – 10.4	6.2 – 9.7			
Ventral scales	33.9 ± 0.2	32.5 ± 0.3	35.6 ± 0.3	33.0 ± 0.3	$F_{1,\,28} = 43.50$, $P < 0.0001$; F > M	$F_{1,\,28} = 13.96$, $P < 0.001$; ST > LT	$F_{1,\,28} = 3.83$, $P = 0.060$
	33 – 35	31 – 34	34 – 37	32 – 35			

Values are expressed as mean ± SE and range. Comparisons are performed by using two-way ANOVA (for neonate SVL and ventral scales) or two-way ANCOVA (for the remaining variables, with neonate SVL as the covariate) with basking opportunity and sex as the factors. F, Females; M, males; LT, basking for 14-h daily; and ST, basking for 10-h daily

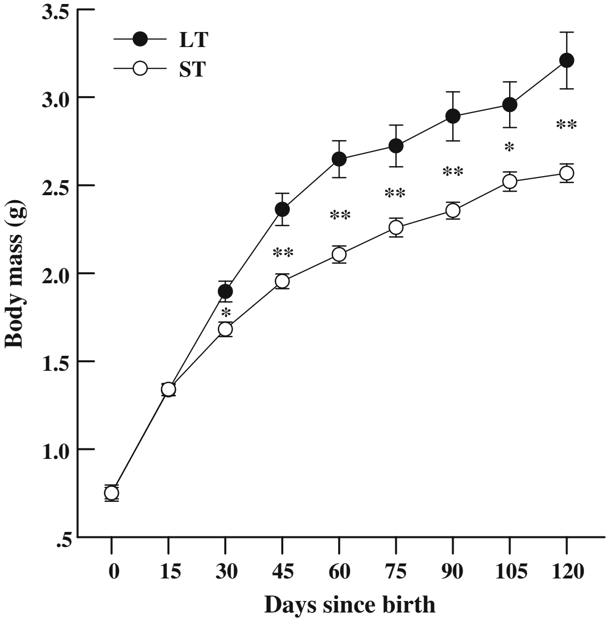

Fig. 3 Mean values (±SE) for early growth of neonates born in the LT (solid dots) and ST (open dots) treatments, with the neonate mass at birth being set at 0.75 g. Differences in body mass between the two treatments, as revealed by ANCOVAs, are denoted by asterisks: $*P < 0.05$; $**P < 0.01$

born in the two treatments did not differ in mass in the first 15-day period, but offspring born in the LT treatment significantly outweighed those born in the ST treatment thereafter (Fig. 3).

Discussion

Our data show that *E. prezwalskii* is among those lacertid lizards that are sexually dimorphic at birth or hatching (Hao et al. 2006 and included references). Male hatchlings or newborns have larger heads but fewer ventral scales than females of the same SVL in all these lizards, whereas other morphological traits differ between sexes in some species but not in others. For example, males have longer tails than females of the same SVL in *E. prezwalskii* (the present study) and *Podarcis muralis* (common wall lizard; Braña and Ji 2000) but not in *Eremias argus* (Mongolian racerunner; Hao et al. 2006) and *Takydromus septentrionalis* (northern grass lizard; Du and Ji 2006). Consistent with studies of other viviparous lizards (Schwarzkopf and Shine 1991; Shine and Harlow 1993; Wapstra 2000; Ji et al. 2006, 2007), the results of this study show that maternal thermal regimes affect gestation length but not litter size in *E. prezwalskii*. Females provided with longer basking opportunity had earlier parturition dates than did females provided with shorter basking opportunity (Table 1). This observation is not surprising since females in the LT treatment had higher average body temperatures, and higher gestation temperatures accelerate embryonic development (Beuchat 1988; Shine and Harlow 1996; Wapstra 2000; Ji et al. 2006, 2007). Of the offspring traits examined, tail length, the number of ventral scales, locomotor performance and postnatal growth were affected by the experimental treatment.

In the following, we discuss our results in the framework of the maternal manipulation hypothesis.

Females shift their thermal preferences when gravid

Thermal preference is not a fixed trait for any reptile species as it can be affected by many extrinsic and intrinsic factors (Lutterschmidt and Hutchison 1997; Rock et al. 2000; Brown and Griffin 2005; Yang et al. 2008). In a wide variety of squamate reptiles, for example, females shift thermal preferences (both upwards and downwards) when gravid (Beuchat 1988; Braña 1993; Daut and Andrews 1993; Andrews and Rose 1994; Mathies and Andrews 1997). Modification of thermoregulatory behavior 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 viviparity in reptiles (Shine 1995, 2006). As in other viviparous lizards such as *Lacerta vivipara* (common lizard; Van Damme et al. 1986), *Sphenomorphus indicus* (brown forest skink; Ji et al. 2006) and *M. multifasciata* (Ji et al. 2007), females selected lower body temperatures when gravid (mean = 30.4°C) than when postpartum (mean = 32.5°C) in *E. prezwalskii*. This result validates one prediction of the maternal manipulation model that females should modify their thermal preferences when gravid.

Given that prolonged gestation lengths at low body temperatures increase reproductive costs associated with decreased survival or future reproduction of females by increasing the time over which these costs are incurred, then why did gravid females of *E. prezwalskii* select lower body temperatures even though higher body temperatures would have been easily attainable on the thermal gradient? Our explanation is that maintaining lower-than-usual body temperatures at about 30°C allows female *E. prezwalskii* to produce good-quality offspring in a relatively short gestation length. Among reptiles, the rate of embryonic development generally increases at an ever-decreasing rate as incubation or gestation temperature increases (Deeming and Ferguson 1991). For example, the mean incubation length is shortened by about 11 days from 27 to 30°C and by about 3 days from 30 to 33°C in *E. argus* (Hao et al. 2006), and the mean gestation length is shortened by about 21 days from 26 to 29°C and by about 5 days from 29 to 32°C in *M. multifasciata* (Ji et al. 2007). Such a pattern of thermal dependence of incubation or gestation length provides an inference that gravid females benefit increasingly less from selecting higher body temperatures in terms of the reduced gestation length. We are currently unaware of the lower and upper thresholds over which temperatures are detrimental to developing embryos in *E. prezwalskii*. However, the result from a recent study of another congeneric lizard (*E. argus*) is that temperatures lower than 24°C and higher than 33°C either dramatically increase embryonic mortality or lead to production of poor-quality offspring (Hao et al. 2006). *Eremias argus* and *E. prezwalskii* are similar ecologically and are sympatric over broad areas in northern China (Zhao 1999). In light of the fact that temperatures optimal for embryonic development are similar among reptiles that are similar in habitat use and/or distribution (Lin et al. 2005 and included references), body temperatures (29.1–32.4°C) selected by gravid females of *E. prezwalskii* should be close to the upper limit of temperatures optimal for embryonic development, allowing production of good-quality offspring in a relatively short gestation length.

As reptiles are ectothermic they cannot maintain less variable body temperatures in thermally variable environments without careful thermoregulation. In the present study, gravid females, non-gravid females and adult males differed from each other in the mean rather than the variance of selected body temperatures. Because body temperatures were not measured over the whole day, we currently have no way of knowing whether gravid females of *E. prezwalskii* thermoregulate more carefully and, thus, maintain more stable body temperatures during the day than non-gravid or male lizards. Females maintain less variable body temperatures when gravid in cold-climate reptiles (Charland and Gregory 1990; Peterson et al. 1993; Charland 1995), and less variable gestation or incubation temperatures have been claimed to enhance offspring fitness in warm-climate reptiles (Webb et al. 2006; Ji et al. 2007; Lin et al. 2007).

Phenotypic traits forged by maternal basking affect offspring fitness

Our data show that the basking opportunities available to a gravid female affect the morphology and performance abilities of her offspring. The functional links through which variation in offspring morphological traits could be translated into variation in the offspring's fitness are currently unknown, but locomotor performance and postnatal or posthatching growth have been well known to be fitness-related (Van Damme et al. 1992; Rhen and Lang 1995; Qualls and Andrews 1999; Braña and Ji 2000). Offspring born in the LT treatment not only performed better in the racetrack but also grew faster than did offspring born in the ST treatment (Figs. 2, 3). These observations suggest that more basking is better in *E. prezwalskii*; that is, the mother may give birth to offspring with enhanced locomotor and growth performances if she basks more. Locomotor performance is of importance for avoiding predators and enhancing foraging success (Bauwens et al. 1995; Miles et al. 1995; Shine 2003), and early growth rates imply either accelerating the age at first reproduction or raising the size at maturity (Galán 1996; Alberts et al. 1997; Braña and Ji 2000). Therefore, our data validate the other main prediction of the maternal manipulation hypothesis, that phenotypic traits determined by maternal thermoregulation should enhance offspring fitness.

It is worth noting that more maternal basking may not be always better. For example, a fourfold increase in maternal basking opportunities in *Eulamprus heatwolei* produced only a 12% decrease in gestation length but resulted in neonates being less active, and in being slower runners (Shine and Harlow 1993). Therefore, for future work, it could be interesting to examine whether maternal basking may differ among reptiles that differ in habitat use and/or distribution.

In summary, our data conform to the main predictions of the maternal manipulation hypothesis: that females should shift selected body temperatures during gestation to provide optimal thermal conditions for developing embryos and that phenotypic traits determined 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 gravid females do not maintain more stable body temperatures than do postpartum females and adult males in *E. prezwalskii*. Our study is the first to demonstrate that the maternal manipulation hypothesis, which may explain selective advantage of viviparity in tropical reptiles, also applies to cold-climate viviparous reptiles.

Acknowledgements The Forestry Bureau of Inner Mongolia Autonomous Region provided a permit for capturing lizards in Wulatehouqi. The experiment complied with the current laws on animal welfare and research in China. We thank Hong An, Guo-Hua Ding, Hong-Liang Lu, Qun-Li Zhang and Wen-Bin Mei for their assistance. Comments by Richard Shine, Daniel Blackburn and one anonymous reviewer substantially improved the earlier version of this paper. This work was supported by grants from National Natural Science Foundation of China (Project No. 30670281), Nanjing Normal University and Hangzhou Normal University.

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