Gestation temperature affects sexual phenotype, morphology, locomotor performance, and growth of neonatal brown forest skinks, *Sphenomorphus indicus*

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We maintained pregnant *Sphenomorphus indicus* under four thermal conditions for the whole gestation period to assess the effects of gestation temperature on offspring phenotypes. Parturition occurred between late June and early August, with females at high body temperatures giving birth earlier than those maintained at low body temperatures. Litter size, litter mass, and postpartum body mass did not differ among treatments, and females with relatively higher fecundity produced smaller offspring. Females gave birth to predominantly female offspring (85.7% of the 14 sexed offspring were females) at 24 °C and to predominantly male offspring (76.5% of the 17 sexed offspring were males) at 28 °C. Females with the opportunity to regulate body temperature produced a mix of sexes that did not differ from equality. Offspring produced in different treatments differed in head size, hind-limb length, and tympanum length, but not in snout-vent length, tail length, body mass, fore-limb length, and eye length. Offspring produced at 28 °C were not only smaller in head size, but also shorter in hind-limb length and tympanum length than those offspring produced at lower temperatures. Offspring produced at 28 °C performed more poorly in the racetrack and grew more slowly than offspring produced in the other three treatments. Taken together, our results show that *S. indicus* might be a temperature-dependent sex determination species and that offspring phenotypes are impaired at high gestation temperatures but maximized at relatively low gestation temperatures. © 2006 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2006, **88**, 453–463.

ADDITIONAL KEYWORDS: offspring phenotype – performance – reproduction – selected body temperature – Sincidae – temperature-dependent sex determination.

INTRODUCTION

Temperature influences organisms in many ways, including development rate, growth, morphology, body size, behaviour, reproduction, and even sexual differentiation in species with temperature-dependent sex determination (TSD) (Bull, 1980; Ratte, 1985; Janzen & Paukstis, 1991; Burggren & Just, 1992; Atkinson, 1994; Huey & Berrigan, 1996; Johnston *et al.*, 1996). Influences of temperature on an organism's fitness-related traits occurring in early ontogenetic stages have greater effects on its subsequent development than do influences occurring in later ontogenetic stages (Desai & Hales, 1997; Lindström, 1999; Lummaa & Clutton-Brock, 2002; Deeming, 2004). For example, in reptiles, thermal environments experienced by embryos can substantially modify offspring phenotypes, and some modifications are permanent or long lasting and can therefore influence an offspring's

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fitness (Webb & Cooper-Preston, 1989; Burger, 1991, 1998; Shine, 1995; Andrews, Mathies & Warner, 2000; Braña & Ji, 2000; Ji *et al.*, 2003).

Unlike embryos of endothermic vertebrates, reptilian embryos of most species can develop successfully over a relatively wide range of temperatures. Within the range of temperatures yielding viable offspring, the rate of embryonic development is greatly accelerated at high temperatures (Vinegar, 1974). However, temperatures maximizing the rate of embryonic development often do not maximize offspring phenotypes (Van Damme *et al.*, 1992; Overall, 1994; Braña & Ji, 2000; Ji & Zhang, 2001; Ji, Qiu & Diong, 2002; Ji *et al.*, 2003; Lin & Ji, 2005). Thus, providing optimal temperatures for developing embryos by the mother herself through thermoregulation, nest-selection or egg-brooding may have great consequences for offspring phenotypes (Shine & Harlow, 1996; Shine *et al.*, 1996; Shine & Elphick, 2001; Webb, Brown & Shine, 2001).

Most work on the phenotypic effects of temperature has been conducted on oviparous reptiles, whereas parallel studies focusing on viviparous species are relatively limited (but see also Beuchat, 1988; Schwarzkopf & Shine, 1991; Shine & Harlow, 1993; Mathies & Andrews, 1997; Swain & Jones, 2000; Wapstra, 2000; Olsson & Shine, 2001; Robert & Thompson, 2001; Lourdais *et al.*, 2004; Wapstra *et al.*, 2004). Unlike oviparous females that never control thermal conditions for their eggs (except for those brooding eggs; Wang, 1966; Shine *et al.*, 1996), viviparous females have the potential to provide optimal temperatures for developing embryos by behavioural thermoregulation. Nonetheless, if ambient temperatures are extreme or fluctuate considerably, viviparous females are unable to regulate their body temperatures to the range suitable for their embryos (Lourdais *et al.*, 2004). Thus, providing pregnant females the opportunity to regulate body temperature may have great consequences for offspring phenotypes.

In the present study, we investigate variation in offspring phenotypes induced by thermal environments experienced by pregnant brown forest skinks (*Sphenomorphus indicus*). This medium-sized [up to 98 mm snout-vent length (SVL)] viviparous skink is widely distributed in the southern part of China, India (Darjeeling and Sikkim), Indochina, and Malay Peninsula. Females produce a single litter per year, with a litter size of three to 11 young (Ji & Du, 2000). Pregnant females were maintained under four thermal conditions so that they could either maintain body temperatures (cloacal Tb) constant at 24 °C and 28 °C or regulate body temperature in the laboratory by exploitation of heat sources available in a thermal gradient or in an enclosure. The body temperatures of 24 °C and 28 °C were, respectively, close to the lower (23.5 °C) and upper (28.3 °C) limits of the central 80%

of body temperatures selected by pregnant females in the laboratory thermal gradient (see below). Some temperatures (up to 36.5 °C) in the enclosure were close to the critical thermal maximum reported for the species (37.6 °C; Ji, Sun & Du, 1997), but brief periods of daily exposure of females to these temperatures would not necessarily increase mortality. The present study has two main objectives: (1) to examine the effects of gestation temperature on sexual phenotype, morphology, locomotor performance, and early growth of offspring and (2) to evaluate the potential links through which phenotypic variation induced by gestation temperature can be translated into differences in fitness.

MATERIAL AND METHODS

COLLECTION AND ANIMAL CARE

We collected 70 skinks on 6–15 May 2003 from a population in Hangzhou (30°16′N, 120°12′E), eastern China, and brought them to our laboratory in Hangzhou, where five skinks were also dissected to confirm that all were ready to ovulate or had just ovulated. The remaining females were maintained under four thermal conditions (thermal treatments) until parturition. In each treatment, females were housed 7–9 in each $100 \times 60 \times 50$ cm (length × width × height) glass cage, of which the bottom was filled with moist soil, debris and grasses to a depth of 10 cm. Mealworms (larvae of *Tenebrio molitor*) and water enriched with vitamins and minerals (Nekton-Rep, Nekton-product) were provided *ad libitum*.

We maintained females in two of the four treatments in two temperature-controlled rooms, thereby maintaining their mean body temperatures at 24 ± 0.3 °C and 28 ± 0.3 °C, respectively. Body temperature was taken for each female using a WMZ-3 electronic thermometer (Shanghai Medical Instrument) to confirm that the mean body temperatures were controlled at the anticipated levels.

The cages holding females in the third treatment (F-treatment) were placed in the backyard of our laboratory. A datalogger (Tinytalk TK-0014, Gemini Pty) programmed to record ambient temperature every 60 min was placed 30 cm above the ground throughout the experiment. The downloaded data showed that ambient temperatures in the backyard varied daily and seasonally, with the mean, the lowest and the highest temperatures being 27.9, 20.9 and 36.5 °C, respectively (Fig. 1).

Females in the fourth treatment (TR-treatment) were maintained in a room where the surrounding temperatures were never higher than 20 °C. A 275-W lightbulb suspended above one end of each cage (20 cm above the cage floor) created a thermal gradient in the
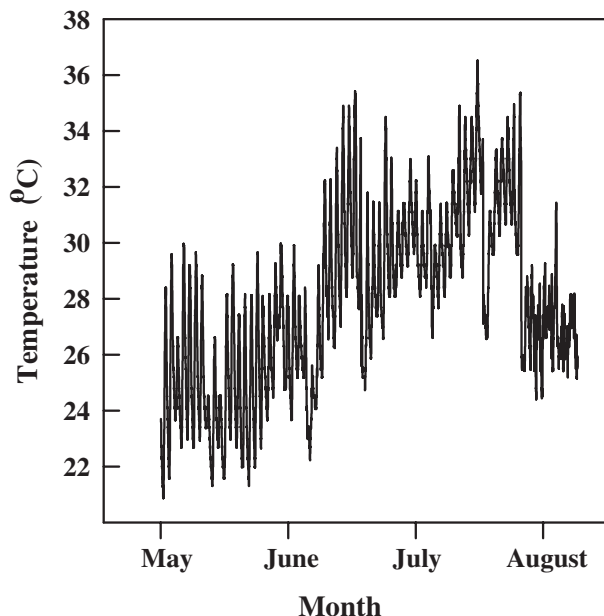
Figure 1. Temporal variation in ambient temperatures experienced by pregnant *Sphenomorphus indicus* maintained in the laboratory enclosure.

range 18–60 °C during the photophase, under a 14 : 10 h light/dark cycle. Body temperatures selected by females ($N = 14$) were in the range 23.0–29.7 °C, with a mean of 25.8 °C (indicative of selected body temperature; Ji *et al.*, 1997). The central 80% of all preferred body temperature readings, which is an estimate of the preference zone for behavioural thermoregulation (Bauwens *et al.*, 1995), included the values in the range 23.5–28.3 °C.

FEMALE REPRODUCTION

The cages were checked daily for newborns as soon as the first female gave birth. Females giving birth during the same period were isolated from each other using $30 \times 30 \times 30$ cm chambers so that offspring could be allocated accurately to the month. Newborns were collected and weighed within a few hours after being produced; body mass, SVL and tail length were taken for each postpartum female. Of the 65 reproducing females, 57 gave birth to young that were all well developed, whereas the remaining females produced litters with various numbers of dead young, stillborns or unfertilized eggs. These abnormal litters, found in each 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 $\log_e$(female SVL).

OFFSPRING PHENOTYPES

A total of 57 neonates, which represented nearly all normal litters, were used to evaluate the effects of gestation temperature on offspring phenotypes. Because locomotor performance is highly sensitive to changes in body temperature in reptiles, we conducted all trials at a body temperature of 30 °C, which was controlled by placing the newborns in an incubator at the correspondent temperature for 30 min prior to testing. Locomotor performance was assessed by chasing down the neonates along a 2-m racetrack with one side transparent, which allowed lateral filmation with a Panasonic NV-DS77 digital video camera. Each neonate was run two times with an approximately 30-min rest between the two successive trials. Two newborns produced in the F-treatment refused to run, and were excluded from analyses. The tapes were later examined with a computer using MGI VideoWave III software (MGI Software Co.) for sprint speed in the fastest 25-cm interval and the maximal distance travelled without stopping (i.e. the maximal length). After examination of locomotor performance, these newborns were killed by freezing to –15 °C for later collection of morphological data. Morphological measurements taken for each newborn included SVL, tail length, head length (from the snout to the anterior edge of tympanum), head width (taken at the posterior end of the mandible), fore-limb (humerus plus ulna) and hind-limb (femur plus tibia) lengths, tympanum length, and eye length.

The remaining newborns were either released to the site where females were collected or were maintained in the laboratory to evaluate the effects of gestation temperature on early (the initial 45-day) growth. To control for any influence of temperature choice of skinks on postembryonic growth that might have resulted from the experimental modification of the thermal environments during embryonic development, we maintained newborns ($N = 90$, marked by painting for future identification) from different treatments under an identical thermal condition. We housed 10–15 newborns in each of six $100 \times 60 \times 50$ cm³ glass cages placed in a controlled temperature room at 26 ± 0.3 °C. The room lights were programmed to create a 12 : 12 h light/dark cycle. Newborns had free access to small-sized mealworms and water enriched with vitamins and minerals in excess. Two individuals died at 7 and 26 days of age, respectively; the remaining newborns were weighed again and sexed by using palpation of hemipenes and histology of neonatal gonads at 45 days of age.

STATISTICAL ANALYSIS

All data were tested for normality using the Kolmogorov–Smirnov test and, for homogeneity of

variances, using Bartlett's test. Log$_e$ transformation was performed when necessary to satisfy the assumptions for using parametric tests. Linear regression analysis, linear correlation analysis, one-way analysis of variance (ANOVA), one-way analysis of covariance (ANCOVA), and repeated-measures ANOVA were used to analyse the corresponding data, when the assumptions for parametric analyses were met. A *G*-test was used to examine whether the sex ratio differed from equality within each treatment. A principal component analysis (varimax rotation) was used to investigate the possible existence of morphological spaces characteristic of offspring from different thermal environments. Tukey's test was used for all multiple comparisons. For all data, all values are presented as mean ± SE, and the significance level is set at $\alpha = 0.05$.

RESULTS

FEMALE REPRODUCTION

Parturition occurred between late June and early August (Fig. 2). The mean interval after capture but before parturition varied from 51.3 days (treatment at 28 °C) to 85.5 days (TR-treatment), and differed significantly among treatments ($F_{3,53} = 200.60$, $P < 0.0001$). Females at higher (average) body temperatures generally gave birth earlier than those at lower body temperatures (Fig. 2). Litter size, litter mass and postpartum mass did not differ among treatments when controlled for differences in female SVL (ANCOVA; all $P > 0.09$). Offspring mass was independent of female SVL within each treatment, and the mean offspring mass did not differ among treatments (ANOVA; $F_{3,53} = 2.12$, $P = 0.108$) (Table 1). When pooling data for the four treatments, offspring mass was found to be negatively correlated with relative

fecundity ($r = -0.37$, $F_{1,55} = 8.51$, $P < 0.005$) (Fig. 3). This result indicates that female *S. indicus* with relative higher fecundity tend to produce smaller young.

MORPHOLOGY OF OFFSPRING

Head size (both length and width), hind-limb length, and tympanum length were significantly affected by gestation temperature, whereas SVL, tail length, body mass, and eye length were not (Table 2). Newborns produced at 28 °C were smaller in head length, head width, hind-limb length, and tympanum length rela-
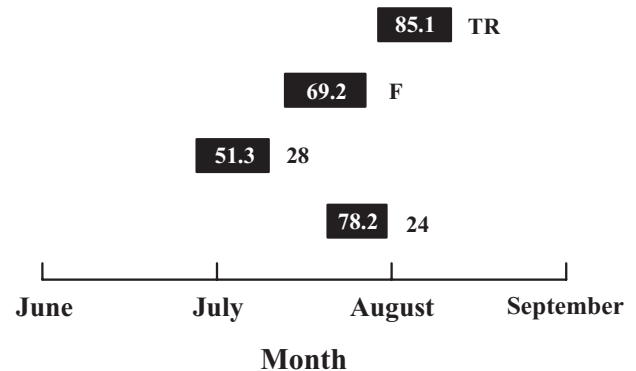

Figure 2. Birth dates of offspring produced by females maintained under different thermal conditions. Thermal treatments: 24 and 28, females whose body temperatures were controlled at 24 °C ($N = 14$) and 28 °C ($N = 12$), respectively; F, females ($N = 17$) maintained in the laboratory enclosure; TR, females ($N = 14$) maintained in the laboratory thermal gradient created by heating lights under an 14 : 10 h light/dark cycle. Numbers in the horizontal bars indicate mean days after capture but before parturition. F, females maintained in the laboratory enclosure; TR, females maintained in the laboratory thermal gradient.

Table 1. Descriptive statistics of female reproductive traits of *Sphenomorphus indicus*

Thermal treatments	24 °C ($N = 14$)	28 °C ($N = 12$)	F ($N = 17$)	TR ($N = 14$)
Snout-vent length (mm)	81.0 ± 0.9 (75.5–87.2)	82.3 ± 1.4 (76.4–90.7)	83.3 ± 1.0 (76.2–88.7)	83.8 ± 1.0 (72.0–90.9)
Tail length (mm)	96.4 ± 10.1 (14.0–137.0)	101.2 ± 7.1 (64.0–104.0)	93.0 ± 6.1 (45.0–134.0)	104.0 ± 4.1 (85.0–133.0)
Postpartum body mass (g)	10.3 ± 0.4 (7.3–14.4)	11.3 ± 0.5 (8.2–14.4)	12.2 ± 0.5 (7.7–17.0)	11.4 ± 0.4 (8.3–13.1)
Litter size	6.7 ± 0.4 (3–9))	7.3 ± 0.4 (4–10)	5.9 ± 0.6 (3–9)	6.8 ± 0.4 (3–9)
Litter mass (g)	3.0 ± 0.2 (1.3–4.0)	2.7 ± 0.3 (1.4–4.4)	3.2 ± 0.2 (1.7–5.1)	3.5 ± 0.2 (1.4–4.9)
Neonatal mass (g)	0.46 ± 0.02 (0.36–0.55)	0.47 ± 0.02 (0.36–0.70)	0.46 ± 0.02 (0.33–0.62)	0.51 ± 0.01 (0.45–0.61)
Relative litter mass	0.30 ± 0.02 (0.18–0.39)	0.25 ± 0.02 (0.14–0.40)	0.28 ± 0.02 (0.18–0.40)	0.30 ± 0.02 (0.17–0.44)

Females that were ready to ovulate or had just ovulated were collected on 6–15 May 2003 from a population in Hangzhou, and were maintained under four temperature regimes until parturition. Values are expressed as mean ± SE and range. F, females maintained in the laboratory enclosure; TR, females maintained in laboratory thermal gradient.
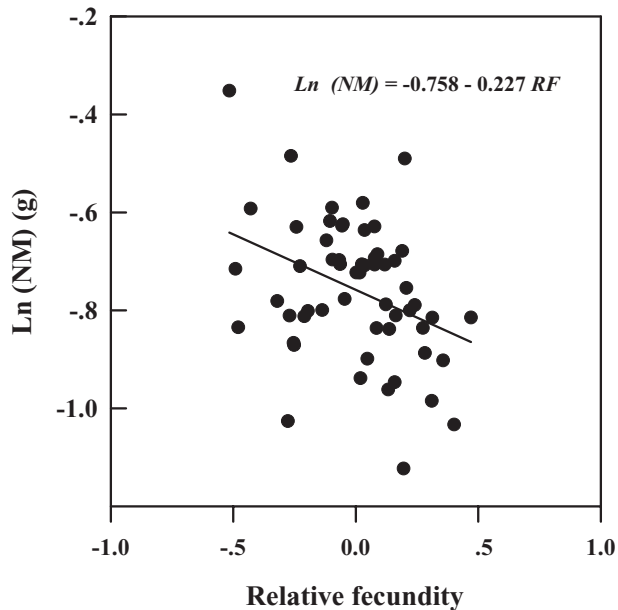
Figure 3. The relationship between neonatal mass and relative fecundity, showing the trade-off between size and number of offspring. All data are log-transformed. The regression equation is also indicated.

tive to SVL than those produced at 24 °C and in the TR-treatment, whereas newborns produced in the F-treatment were intermediate with respect to these thermally plastic morphological traits (Table 2).

A principal component analysis resolved two components (eigenvalues ≥ 1) from nine SVL-free neonatal variables, accounting for 47.4% of variation in the original data (Table 3). The first component (28.6% of variance explained) had high positive loading for SVL-free values of head length and tympanum length. Because tympanum length was positively correlated with head length ($r = 0.66$, $F_{1,55} = 41.92$, $P < 0.0001$), the high scores in the first axis should represent a comparatively elongated head. The second axis (18.8% of variance explained) mainly represented wet body mass. Neonates differed significantly among treatments in their scores on the first axis (ANOVA, $F_{3,52} = 7.19$, $P < 0.0004$; 24 °C[a], 28 °C[b], F-treatment[ab], TR-treatment[a], Tukey's test; a > b), but not on the second axis ($F_{3,52} = 1.91$, $P = 0.140$) (Fig. 4).

LOCOMOTOR PERFORMANCE OF OFFSPRING

SVL, tail length, and body mass of offspring were not correlated with the examined variables of locomotor performance (all P > 0.05), probably because of the limited range of offspring sizes. One-way ANOVAs revealed that maximum length ($F_{3,51} = 2.97$, $P = 0.040$) and sprint speed ($F_{3,51} = 3.68$, $P < 0.02$) were both affected by gestation temperature. The maximum

length was longest for treatment at 24 °C, intermediate in F-treatment and TR-treatment and shortest for treatment at 28 °C (Fig. 5). Sprint speed was fastest for treatment at 24 °C and TR-treatment, intermediate in F-treatment and slowest for treatment at 28 °C (Fig. 5).

SEXUAL PHENOTYPE AND EARLY GROWTH OF OFFSPRING

Skinks at 45 days of age could be sexed accurately by using palpation of hemipenes and histology of gonads. Females at 24 °C gave birth to predominantly female offspring ($G = 7.92$, d.f. = 1, $P < 0.005$), and females at 28 °C gave birth to predominantly male offspring ($G = 5.02$, d.f. = 1, $P < 0.03$). The sex ratio was exactly equal to 1 : 1 in F-treatment, and did not differ from equality in the TR-treatment ($G = 0.33$, d.f. = 1, $P > 0.50$) (Fig. 6).

Across the thermal treatments, changes in mass in the first 45-day period [$\log_e$(mass at day 45) – $\log_e$(neonate mass)] were not correlated with offspring mass at parturition (all $P > 0.930$). Thus, a repeated-measures ANOVA (with gestation temperature as between subject factor and mass at day 0 and 45 as within subject factor) was used to examine the effect of gestation temperature on mass gain at 45 days. Growth was evident in all treatments (highly significant effect for the within subject effect; $F_{1,84} = 803.29$, $P < 0.0001$); the effect of gestation temperature on mass gain was significant ($F_{3,84} = 6.92$, $P < 0.001$), with newborns produced at 28 °C growing much more slowly than newborns produced in the other three treatments (Fig. 7).

DISCUSSION

High temperatures result in faster embryonic development and, thus, earlier parturition dates in *S. indicus* (Fig. 2). Given that all females had decided their litter sizes when they collected, it is not surprising that litter size did not vary among treatments. However, because embryos developed under very different thermal conditions, the consistency of offspring size across the thermal treatments could be an interesting finding, presumably suggesting that offspring size is relatively less sensitive to variation in gestation temperature. In the present study, gestation temperature significantly affected several fitness-related offspring traits, including sexual phenotype, morphology, locomotor performance, and growth. These results provide the following three inferences.

SPHENOMORPHUS INDICUS MIGHT BE A TSD SPECIES

The sex ratio was highly biased to male offspring at 28 °C and to female offspring at 24 °C in *S. indicus*

Table 2. Morphological phenotypes of *Sphenomorphus indicus* offspring produced by females maintained under different thermal conditions

Hatchling traits	Thermal treatments				Statistical analyses and the results of Tukey's multiple comparisons
	24 °C ($N = 11$)	28 °C ($N = 13$)	F ($N = 18$)	TR ($N = 15$)	
Snout-vent length (mm)	29.4 ± 0.3 (27.9–31.4)	29.4 ± 0.3 (27.8–31.7)	28.9 ± 0.4 (26.7–32.0)	30.1 ± 0.2 (28.8–31.8)	2.66[NS]
Tail length (mm)	39.7 ± 0.5 (38.0–44.1)	39.9 ± 0.6 (37.5–35.4)	37.8 ± 0.9 (30.8–42.7)	40.3 ± 0.4 (38.1–42.9)	1.78[NS]
Wet body mass (mg)	464.5 ± 19.4 (365.6–578.6)	496.0 ± 27.3 (395.8–699.8)	454.1 ± 17.9 (343.8–623.0)	504.6 ± 13.3 (413.9–627.9)	1.04[NS]
Dry body mass (mg)	90.5 ± 4.6 (64.4–114.1)	98.7 ± 7.4 (61.6–164.6)	94.7 ± 6.2 (60.3–156.6)	109.0 ± 4.0 (90.4–148.1)	1.10[NS]
Head length (mm)	7.10 ± 0.05 (6.79–7.41)	6.97 ± 0.05 (6.74–7.47)	6.96 ± 0.06 (6.53–7.43)	7.22 ± 0.04 (6.99–7.49)	7.16***, 24[a,b], 28[c], F[b,c], TR[a]
Head width (mm)	4.81 ± 0.04 (4.64–5.01)	4.66 ± 0.03 (4.52–4.97)	4.74 ± 0.04 (4.54–4.98)	4.91 ± 0.03 (4.73–5.27)	8.24***, 24[a], 28[b], F[a], TR[a]
Fore-limb length (mm)	7.39 ± 0.07 (6.91–7.72)	7.32 ± 0.07 (6.89–7.81)	7.18 ± 0.10 (6.28–7.92)	7.63 ± 0.07 (6.99–7.96)	2.68[NS]
Hind-limb length (mm)	9.82 ± 0.11 (9.08–10.30)	9.79 ± 0.12 (8.97–10.71)	9.38 ± 0.15 (8.19–10.48)	10.08 ± 0.08 (9.49–10.49)	3.81*, 24[a,b], 28[b], F[a,b], TR[a]
Tympanum length (mm)	1.05 ± 0.02 (0.89–1.16)	0.98 ± 0.01 (0.93–1.06)	1.00 ± 0.01 (0.85–1.06)	1.07 ± 0.02 (0.97–1.29)	4.43**, 24[a], 28[b], F[a,b], TR[a]
Eye length (mm)	2.38 ± 0.03 (2.23–2.53)	2.33 ± 0.02 (2.26–2.44)	2.34 ± 0.03 (2.01–2.54)	2.40 ± 0.02 (2.27–2.58)	0.83[NS]

Values are expressed as mean ± SE and range. Snout-vent length is the covariate in all ANCOVA models. Symbols immediately after *F*-values represent significant levels: NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Means corresponding to thermal treatments with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$, a > b > c). F, females maintained in the laboratory enclosure; TR, females maintained in laboratory thermal gradient.

Table 3. Loading of the first two axes of a principal component analysis on nine variables of neonate traits

	Factor loading	
	PC1	PC2
Wet mass	−0.062	0.726*
Dry mass	0.172	0.595
Tail length	0.256	0.394
Head width	0.425	0.095
Head length	0.788*	0.178
Fore-limb length	0.667	0.462
Hind-limb length	0.555	0.510
Eye length	0.575	−0.355
Tympanum length	0.771*	−0.114
Variance explained	28.6%	18.8%

Size effects are removed in all cases by using residuals from the regressions on snout-vent length of neonates. All data were log-transformed. *Variables with the main contribution to each factor.
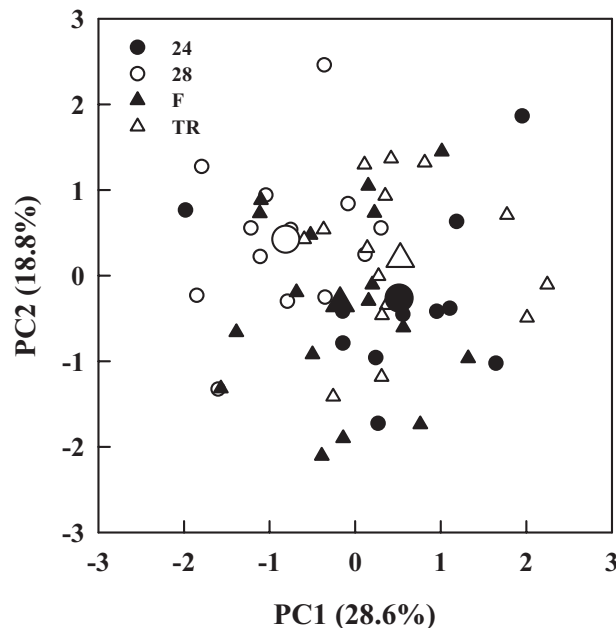

Figure 4. Positions of offspring produced by females maintained under different temperature regimes in the space defined by the first two axes of a principal component analysis based on nine size-adjusted morphological variables of offspring, on which size effects were removed by using residuals from the regressions on offspring snout-vent length. Enlarged symbols show the mean values of scores on the first two axes. F, females maintained in the laboratory enclosure; TR, females maintained in the laboratory thermal gradient.
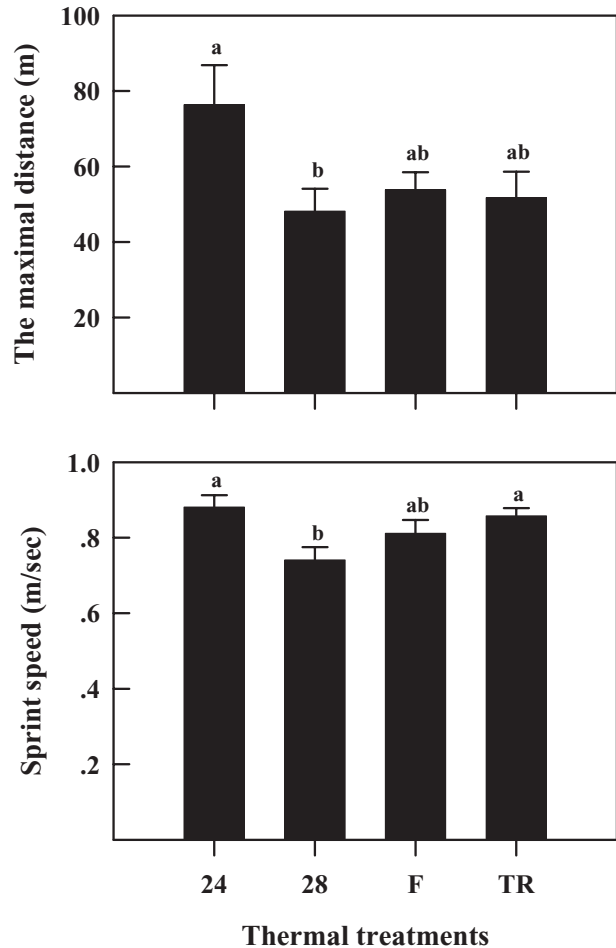
Figure 5. Locomotor performance (upper: the maximal distance travelled without stopping; lower: spring speed) of newborn *Sphenomorphus indicus* produced by females maintained under different thermal conditions. Values are expressed as mean ± SE. Means with different superscripts differ significantly (one-way ANOVA, Tukey's test, $\alpha = 0.05$, a > b). 24: $N = 11$; 28: $N = 13$; F: $N = 16$; TR: $N = 15$. F, females maintained in the laboratory enclosure; TR, females maintained in the laboratory thermal gradient.
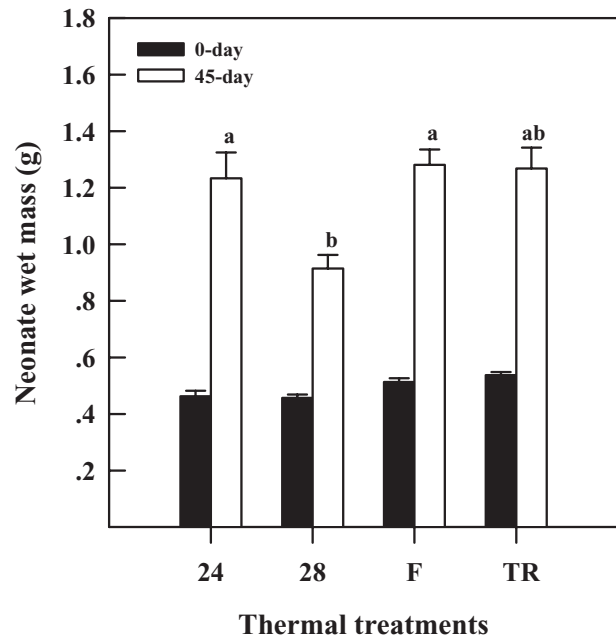
(Fig. 6). Because the sexed neonates were randomly sampled from each normal litter (100% offspring were alive) and 97.8% (88/90) of them survived at 45 days of age when they were sexed, the differential sex-specific mortality is unlikely to be the alternative cause for the highly-biased sex ratios at 24 °C and 28 °C. Thus, *S. indicus* might be a TSD species. Females in the F- and TR-treatments stabilized the sex ratio of offspring to 1 : 1 (Fig. 6), matching the sex ratio in the natural population from which our females were collected (Ji & Du, 2000). Interestingly, the mean of body temperatures selected by the females in the TR-treatment was 25.8 °C, which is approximately half way between 24 °C and 28 °C. In TSD species, shifts in sex ratio
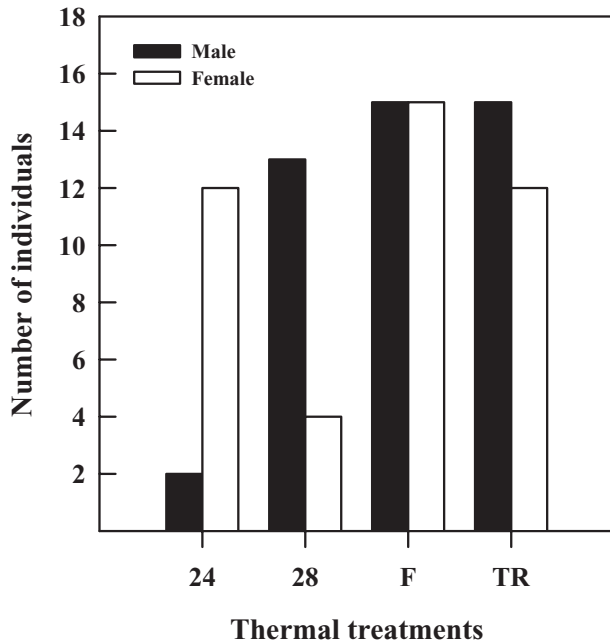
Figure 6. Influence of gestation temperature on the sex ratios of offspring. Females whose mean body temperature was controlled at 24 °C gave birth to predominantly female offspring; females whose mean body temperature was controlled at 28 °C gave birth to predominantly male offspring; females maintained in the laboratory enclosure (F-treatment) and in the laboratory thermal gradient (TR-treatment) gave birth to offspring for which the sex ratio did not differ from equality.

Figure 7. Influence of gestation temperature on early growth of offspring. Values are expressed as mean ± SE. Means with different superscripts differ significantly (repeated-measures ANOVA, Tukey's test, α = 0.05, a > b). 24: $N = 14$; 28: $N = 17$; F: $N = 30$; TR: $N = 27$. F, females maintained in the laboratory enclosure; TR, females maintained in the laboratory thermal gradient.

with an increase in incubation or gestation temperature should be continuous rather than dichotomous (Bull, 1980; Janzen & Paukstis, 1991; Valenzuela & Lance, 2004; Wapstra *et al.*, 2004), and it is expected that female *S. indicus* at body temperatures around 26 °C should produce offspring of both sexes. The result that females in the TR-treatment produced offspring of both sexes presumably provides evidence for this expectation.

TSD provides viviparous females with the potential to select neonatal gender (Robert & Thompson, 2001; Wapstra *et al.*, 2004), but the evolution of the TSD pattern in the viviparous species remains unclear. Under the assumption that birth date might influence adult body size in species of which reproductive success is more tightly linked in one of the sexes, Wapstra *et al.* (2004) hypothesized that the difference in TSD pattern would relate to sex differences in reproductive success resulting from sex differences in birth date. However, this speculation is unlikely to be true in *S. indicus*. For example, although *S. indicus* and *Eulamprus tympanum* (water skink; Robert & Thompson, 2001) have the same 'TSD' pattern, reproductive success is more tightly linked to body size in adult

females in *S. indicus* (Ji & Du, 2000) but, probably, in adult males in *E. tympanum* (Wapstra *et al.*, 2004). By contrast, *S. indicus* and *Niveoscincus ocellatus* (spotted skink, Wapstra *et al.*, 2004) have the opposite 'TSD' pattern, although reproductive success is more tightly linked to body size in adult females in both species (Ji & Du, 2000; Olsson *et al.*, 2002).

OFFSPRING PHENOTYPES ARE IMPAIRED AT HIGH GESTATION TEMPERATURES

Offspring produced at 28 °C had smaller heads relative to SVL than those offspring produced in the other three treatments (Table 2). A larger head is associated with an increased ability of a predator to eat larger prey (Schoener *et al.*, 1982; Barden & Shine, 1994). Moreover, according to optimal foraging models, a predator tends to increase the rate of net energy intake by consuming larger prey items to maximize net energy gain. Thus, any relative decrease in head size may potentially decrease an offspring's ability to feed larger prey.

Offspring produced at 28 °C performed more poorly in the racetrack than those offspring produced in other three treatments (Fig. 5). It is worth noting the consistency of results for morphometric and perfor-

mance traits, which allows us to examine the possibility that performance (e.g. locomotor performance) could be indirectly influenced by gestation temperature through its effect on morphology. For example, the reduced hind-limb length in offspring produced at 28 °C might have a detrimental effect on locomotor performance, because hind-limb length is assumed to be associated with locomotor performance in lizards (Miles, 1994; Bauwens *et al.*, 1995). Poor locomotor performance of offspring produced at 28 °C could also reflect behavioural limitations. For example, the shorter maximal distance travelled without stopping in these offspring is clear a contributing factor to their decreased sprint speed because the traits are positively correlated ($r = 0.42$, $F_{1,53} = 11.25$, $P < 0.002$). High gestation temperatures also exert an adverse effect on early growth of *S. indicus*, as indicated by offspring produced at 28 °C growing more slowly than those offspring produced in the other three treatments (Fig. 7).

The mean ambient temperature in the F-treatment was 27.9 °C and females in the treatment had ample opportunities for exposure to extremely high temperatures (to 36.5 °C) close to the critical thermal maximum (37.6 °C; Ji *et al.*, 1997). However, offspring produced in the treatment were more close to those produced in the two lower temperature treatments than to those produced at 28 °C, presumably because the detrimental effects of extremely high ambient temperatures were buffered to some extent through maternal thermoregulation. Considering the 1 : 1 sex ratio and birth dates of offspring, we speculated that the overall mean body temperature in the F-treatment was most probably maintained at approximately 26 °C. Overall, phenotypic traits of offspring produced in the F-treatment were good but not optimal, presumably because of exposure of embryos to extremely high temperatures during the period when females could not control over their body temperatures.

OFFSPRING PHENOTYPES ARE MAXIMIZED AT LOW GESTATION TEMPERATURES

Females at 24 °C produced well-developed offspring with the best performance in the racetrack (Fig. 5). This finding indicates that offspring phenotypes can be optimized at low gestation temperatures in *S. indicus*. However, the benefit of completing embryonic development at low temperatures can be offset by the biased sex ratio and the increased probability of embryonic mortality due to the prolonged gestation period. Accordingly, we expect that pregnant *S. indicus* in nature should regulate body temperature to a range within which they can compromise birth dates, population sex ratios and offspring phenotypes to a large extent. Most probably, the mean value (25.8 °C)

of body temperatures selected by pregnant *S. indicus* in the laboratory thermal gradient falls within this range.

The mean value of body temperatures selected by pregnant *S. indicus* is much lower than that (31.2 °C) reported for *Eumeces chinensis* (Ji *et al.*, 1995), an oviparous skink common in Hangzhou but using open habitats. In *E. chinensis*, the detrimental effects of incubation temperature on hatchling phenotypes cannot be detected until eggs are incubated at temperatures close to 32 °C (Ji & Zhang, 2001). Unlike *E. chinensis*, *S. indicus* is restricted to forestry regions where ambient temperatures are rarely higher than 30 °C. Thus, lower selected body temperatures also reflect that *S. indicus* is a species using cold habitats. This claim can be indirectly confirmed by the observations that the temperature of 28 °C does not have an adverse effect on offspring phenotypes in nearly all species of lizards using open or warm habitats (Lin & Ji, 1998; Ji & Zhang, 2001; Pan & Ji, 2001; Ji *et al.*, 2002). The result that temperatures optimal for embryonic development are lower in species using cold habitats has also been reported for snakes such as *Xenochrophis piscator* (Ji *et al.*, 2001) and *Deinagkistrodon acutus* (Lin & Ji, 2005).

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