

Relatively high but narrow incubation temperatures in lizards depositing eggs in warm and thermally stable nests*

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Abstract We used the Reevese's butterfly lizard *Leiolepis reevesii* as a model animal to test the hypothesis that incubation temperatures should be relatively high but narrow in lizards depositing eggs in warm and thermally stable nests. Eggs were incubated under three constant (27, 30 and 33°C) and one fluctuating temperature regimes. Hatchability was highest at 30°C and lowest at 27°C. The mean rather than the variance of temperatures affected incubation length, and the mean incubation length was 101.1 days at 27°C, 69.6 days at 30°C and 55.3 days at 33°C. Incubation temperature did not affect the sexual phenotype of hatchlings. Hatchlings from different treatments showed little variation in morphological phenotypes, but differed significantly in locomotor phenotypes. Hatchlings incubated at 27°C performed much more poorly in the racetrack than did hatchlings from the other three treatments, and the variance of incubation temperatures negatively affected sprint speed of hatchlings. The incubation temperatures of 27°C and 33°C supported egg incubation, but these two temperatures were not ideal. The range of temperatures suitable for incubation of *L. reevesii* eggs presumably lies between the range of nest temperatures varying most frequently from 28°C to 32°C. Compared with results reported for other squamate reptiles either from cool habitats or from warm habitats but depositing eggs in shallow nests, temperatures suitable for egg incubation are relatively high but narrow in *L. reevesii*. Thus, our data validate the above hypothesis [Acta Zoologica Sinica 53 (3): 437–445, 2007].

Key words Reptilia, Agamidae, *Leiolepis reevesii*, Egg incubation, Hatching success, Hatchling phenotype, Locomotor performance

产卵于温暖且热稳定巢内的蜥蜴具有相对较高但较窄范围的孵化温度*

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摘要 作者用蜡皮蜥 (*Leiolepis reevesii*) 为模型动物, 检验产卵于温暖且热稳定巢内的蜥蜴应有相对较高但较窄的孵化温度的假设。卵在三个恒定温度 (27、30 和 33°C)、一个波动温度处理下孵化。温度的平均值而非方差影响孵化期, 27、30 和 33°C 的平均孵化期分别为 101.1、69.6 和 55.3 d。幼体性别不受孵化温度影响。不同处理孵出的幼体仅有稍许形态差异, 但运动表现差异显著。27°C 孵出幼体在跑道上的表现比其它处理孵出幼体差。卵能在 27°C 和 33°C 下孵化, 但这两个孵化温度并不适宜。蜡皮蜥适宜的孵化温度范围可能处于最频繁的巢温变化范围 (28°C–32°C) 内。与其它在低温生境或温暖生境但产卵于浅巢的有鳞类爬行动物相比较, 蜡皮蜥有相对较高但较窄适宜的卵孵化温度。因此, 作者的数据支持上述假设 [动物学报 53 (3): 437–445, 2007]。

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关键词 爬行纲 鬣蜥科 蜡皮蜥 卵孵化 孵化成功率 幼体表型 运动表现

Thermal environments experienced by reptilian embryos affect not only the rates of embryonic development but also a number of offspring phenotypes, including size, morphology, behavior, performance, and gender in species with temperature-dependent sex determination (see reviews by Birchard, 2004; Deeming, 2004; Valenzuela, 2004). Female reptiles have the potential to control offspring phenotypes by manipulating nest (via nest-site selection) or body (via behavioral thermoregulation) temperatures (Overall, 1994; Shine and Harlow, 1996; Shine et al., 1997a, b; Wapstra et al., 2004). Oviparous females do not control thermal conditions of oviposited eggs (except for those brooding eggs; Wang, 1966; Vinegar et al., 1970; Slip and Shine, 1988; Shine et al., 1997a, b) and, as such, the maternal selection of nest site may have profound effects on offspring phenotypes. The thermal environments within the natural nest are primarily dependent upon environmental temperatures and the net radiation exchange at the nest site over time (Ackerman and Lott, 2004). In species not brooding eggs, the range of temperatures over which successful egg incubation takes place is believed to be primarily dependent upon the general thermal conditions in natural nests (Lu et al., 2006). The range of successful incubation temperatures for any individual species can be estimated by incubating eggs under different temperature regimes, because prolonged exposure of eggs to temperatures outside this range has detrimental effects on embryonic survivorship and/or offspring phenotypes (see reviews in Birchard, 2004).

Compared to avian eggs, reptilian eggs can be incubated over a relatively wide range of temperatures (Birchard, 2004; Booth, 2004). Within squamate reptiles, the range of successful incubation temperatures differs among species from thermally different habitats. For example, eggs can be incubated successfully at relatively low temperatures in species such as the five-paced pit-viper *Deinagkistrodon acutus* (Lin et al., 2005) and the slender forest skink *Scincella modesta* (Lu et al., 2006) from cool habitats, but at relatively high temperatures in species such as the Chinese cobra *Naja atra* (Ji and Du, 2001) and the northern grass lizard *Takydromus septentrionalis* (Lin and Ji, 1998) from warm habitats. It is also likely that there are differences within and among species that have wide geographic distribution. For example, eggs can be incubated at generally cooler temperatures in species having a more northerly or high elevation distribution than in those having a more southerly or low elevation distribution (Birchard, 2004). In the Chinese skink *Eumeces chinensis*, eggs from a more northerly population can be incubated within a relatively wide range of temperatures when compared to those from a more southerly population, primarily because thermal

environments in the former population are more variable (Ji et al., 2002a).

The Reeves's butterfly lizard *Leiolepis reevesii* is a large sized [to 135 mm snout-vent length (SVL)] oviparous agamid lizard that is distributed exclusively in southern China (Hainan, Guangdong and Guangxi) and Vietnam (Lin et al., 2004). The lizard inhabits sunny sandy coastal regions covered with scattered grass patches. Females lay a single clutch of 2 – 8 pliable-shelled eggs per breeding season (April – July) in relatively deep nests (to 800 mm below the ground surface) where temperatures rarely vary outside the range of 28°C – 32°C (Fig. 1). The lizard is therefore an ideal model system to test the hypothesis that successful incubation temperatures are relatively high but narrow in species depositing eggs in warm and thermally stable nests. We estimated the range of suitable incubation temperatures for the lizard by comparing data on hatching success, morphological phenotypes and locomotor performance of hatchlings derived from egg incubated under three constant (27, 30 and 33°C) and one fluctuating temperature regimes. Data from this study also allow us to address three questions: (1) does exposure of eggs of *L. reevesii* to temperatures outside the range of 28°C – 32°C have any adverse effects on hatching success and hatchling phenotypes? (2) what hatchling phenotypes are more likely affected by incubation temperature? (3) does the variance of incubation temperatures affect incubation length and hatchling phenotypes?

1 Materials and methods

1.1 Collection and animal care

A total of 35 gravid females (> 86 mm SVL) were collected between late April and early July 2006 from Ledong (18°45'N, 109°10'E), Hainan, southern China. Females were brought to our laboratory in Wuzhishan, where 5 – 6 individuals were housed together in one 1 000 mm × 600 mm × 600 mm (length × width × height) communal cage, which had a substrate consisting of 200 mm depth sand, pieces of clay tile and grasses. The communal cages were placed in a room where air temperatures were controlled at 28 (± 0.5)°C. A 100-W light bulb, suspended at one end of each cage, created a thermal gradient ranging from ambient room temperature to 55°C for 14 h to allow thermoregulation during the photophase. Females were fed mealworms *Tenebrio molitor*, field-captured grasshoppers and water enriched with vitamins and minerals. Females with shelled oviductal eggs were removed from the communal cages, and housed individually in 400 mm × 400 mm × 500 mm egg-laying cages with 100 mm depth sand and a 20-W spotlight mounted in each cage to allow thermoregulation.

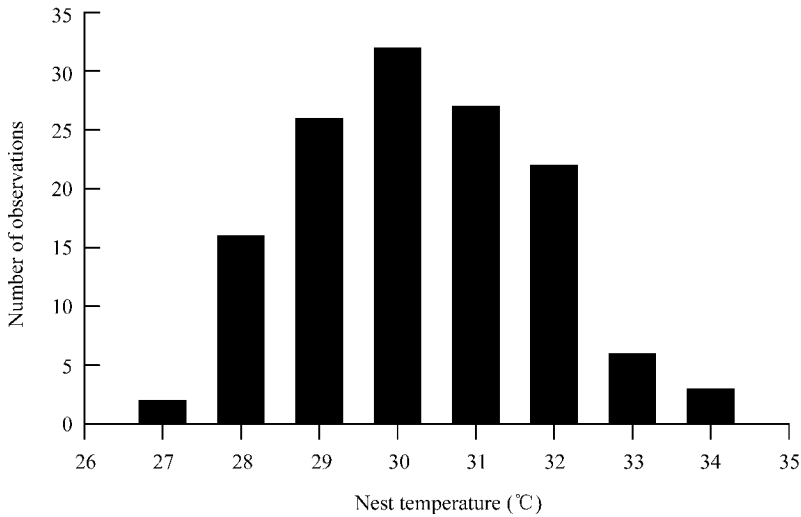


Fig.1 Frequency distributions of nest temperatures measured between May and July 2006 when we studied the nest characteristics of *L. reevesii*

Data presented in the figure were collected from the nests occupied by gravid females or having deposited eggs ($n = 134$). In no case was the temperature of any individual nests measured repeatedly.

1.2 Egg collection and incubation

Eggs were collected, measured (for length and width) and weighed no later than three hours after oviposition, thereby avoiding any uncertainty about the initial mass due to loss or gain of water. The viability of freshly laid eggs was judged by the presence of a small embryonic disc using a spotlight. Post-oviposition females were measured for SVL and tail length and weighed before they were released at the sites where they were originally collected. Twelve freshly laid eggs sampled randomly from 12 clutches were dissected for identification of embryonic stage at oviposition. A total of 101 eggs from 30 clutches were assigned systematically to one of the four thermal conditions (thermal treatments), such that eggs from any one clutch were distributed as equally as possible among treatments.

Eggs were incubated individually in covered plastic jars (50 ml) containing known amounts of vermiculite and distilled water at approximately -220 kPa water potential (1 g dried vermiculite /1 g water; Ji and Braña, 1999). Eggs were half-buried lengthwise in the incubating substrate, with the surface near the embryo exposed to air inside the jar. Jars were weighed on alternate days, and distilled water was added evenly into substrates when necessary to compensate for evaporative losses and water absorbed by eggs, thereby maintaining the substrate water potential constant.

A total of 76 jars were assigned one of the three incubators (Sheldon MFG Inc, USA), with incubation temperatures set at 27, 30 and 33 (± 0.3)°C, respectively. The lowest and highest constant temperatures were chosen because these two temperatures were approximately 1°C outside the range of usual nest temperatures (28°C – 32°C; Fig.1). We moved jars among the shelves in the incubator daily according to a predetermined schedule to

minimize any effects of thermal gradients inside the incubator.

The remaining 25 jars (hereafter the Ft treatment) were placed in a room where air temperatures fluctuated naturally (Fig.2). A Tinytalk datalogger (Gemini Pty, Australia) programmed to record temperature every one hour was placed in the room throughout the incubation period. The mean, minimum and maximum temperatures experienced by individual eggs in the Ft treatment varied from 27.0°C – 28.4°C, 22.3°C – 23.0°C and 32.1°C – 34.1°C, respectively (Table 1). Because constant temperature incubation is rare under natural circumstances, this treatment allows us to examine the effects of thermal fluctuations on hatchability and offspring phenotypes.

Table 1 The thermal environments experienced by eggs ($n = 19$) of *L. reevesii* incubated at fluctuating temperatures

	Mean	SE	Range
Mean temperature (°C)	27.6	0.1	27.0 – 28.4
Minimum temperature (°C)	22.4	0.1	22.3 – 23.0
Maximum temperature (°C)	33.5	0.2	32.6 – 34.1
Thermal variance	3.49	0.08	2.96 – 3.96

Minimum and maximum temperatures: the minimum and maximum temperatures ever experienced by individual incubating eggs.

1.3 Incubation length and hatchling phenotypes

The duration of incubation, measured as the number of days to pipping, was recorded for each egg, and wet body mass was taken for each hatchling. All hatchlings were used to evaluate the effects of incubation temperature on locomotor performance on the day of hatching. We conducted all locomotor trials at the body temperature of 30°C, which was controlled by placing hatchlings in an

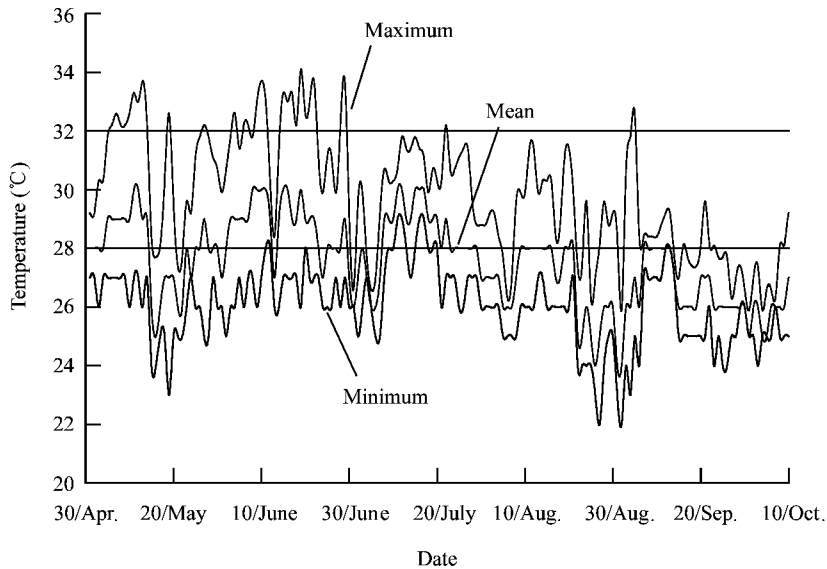


Fig.2 Temporal variation in daily minimum, mean and maximum temperatures recorded by the Tinytalk datalogger placed in a room where the fluctuating temperature experiment was conducted

Eggs of *L. reevesii* deposited in natural nests are less frequently exposed to temperatures higher than 32°C (the upper horizontal line) or lower than 28°C (the lower horizontal line).

incubator at the correspondent temperature for a minimum of 30 min prior to testing. Locomotor performance was assessed by chasing hatchlings along a 2-m racetrack with one side transparent, which allowed videoing with a Panasonic NV-DS77 digital video camera. The tapes were later examined with a computer using MGI VideoWave III software (MGI Software Co., Canada) for sprint speed in the fastest 15-cm interval and the maximum distance traveled without stopping (hereafter the maximum distance).

After examination of locomotor performance, hatchlings were euthanized by freezing to -15°C for later collection of morphological data. Morphological measurements taken for each hatchling 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 tympanum), head width (taken at the posterior end of the mandible), fore-limb length (humerus plus ulna) and hind-limb length (femur plus tibia). Hatchlings were sexed by pressing on both sides of the tail base using forceps for the presence or absence of hemipenes; the presence of hemipenes allowed unequivocal sex assignment of males (Ji and Braña, 1999).

1.4 Statistical analyses

All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Bartlett test). Parametric analyses were used to analyze data when the assumptions for these analyses were met; otherwise, nonparametric analyses were used. Values are presented as Mean \pm SE, and the significance level is set at $\alpha =$

0.05.

2 Results

2.1 Hatching success and incubation length

All of the 12 embryos identified at oviposition were at stage 30 in the Dufaure and Hubert's (1961) developmental series. Mean values for initial egg mass did not differ among the four treatments (One-way ANOVA; $F_{3, 68} = 0.40$, $P = 0.754$). Embryonic mortality differed among the four treatments (G -test; $G = 11.71$, $df = 3$, $P < 0.01$), with hatching success being highest at 30°C and lowest at 27°C (Table 2). Most (11/14) dead embryos in the 27°C treatment were at stage 39–40; dead embryos in the other three treatments were rarely (2/15) at these very late embryonic stages. Incubation length, which was not correlated with initial egg mass within each treatment (all $P > 0.347$), differed among the four treatments (Kruskal-Wallis test; $H_{3, n=72} = 65.41$, $P < 0.0001$). The mean incubation length was shortened by 31.5 days from 27°C to 30°C , and 14.3 days from 30°C to 33°C (Table 2). A simple linear correlation analysis revealed that incubation length was negatively correlated with both the mean ($r = -0.92$, $t = 9.75$, $df = 17$, $P < 0.0001$) and the variance ($r = -0.75$, $t = 4.74$, $df = 17$, $P < 0.0002$) of incubation temperatures in the Ft treatment, whereas a partial correlation analysis revealed that incubation length was actually not correlated with the thermal variance when holding the thermal mean constant ($r = 0.19$, $t = 0.77$, $df = 16$, $P = 0.451$).

Table 2 Effects of incubation thermal environments on duration of incubation, hatching success, sex ratio and abnormality of hatchlings

Thermal treatments (°C)	Number of incubated eggs	Duration of incubation (d)	Hatching success (%)	Sex ratio (♀/♂)	Abnormality (%)
Ft	25	87.1 ± 1.4 (75.9 – 95.6)	76.0 (19/25)	9/10	4.0 (1/25)
27	25	101.1 ± 1.1 (94.3 – 105.9)	44.0 (11/25)	4/7	0 (0/25)
30	28	69.6 ± 0.4 (66.6 – 73.1)	89.3 (25/28)	13/12	10.7 (3/28)
33	23	55.3 ± 0.7 (50.6 – 62.2)	73.9 (17/23)	5/12	0 (0/23)

Data on duration of incubation are expressed as Mean ± SE (range). Ft: the fluctuating temperature treatment.

2.2 Hatchling phenotypes

One hatchling in the Ft treatment and three in the 30°C treatment exhibited either tail or trunk malformation, but the frequency of deformity was independent of treatments ($G = 6.20$, $df = 3$, $P > 0.10$) (Table 2). The sex ratio of hatchlings did not differ significantly among the four treatments ($G = 1.44$, $df = 3$, $P > 0.50$) (Table 2).

Preliminary two-way ANOVAs (with sex and thermal treatment as the factors) or ANCOVAs (with initial egg mass as the covariate) did not reveal between-sex differences in all examined hatchling phenotypes (all $P > 0.25$), so we pooled data for both sexes. Hatchlings from different thermal treatments differed in abdomen length and head width but not in other examined morphological phenotypes (Table 3). The first two components resolved by a principal component analysis from eight size (initial egg mass)-free hatchling variables accounted for 76.8% of variation in original data (Table 4). The first component (46.4% variance explained) had high positive loading for size-free values of head length, head width, fore-limb length and hind-limb length, and the second component (30.4% variance explained) had high positive loading for size-free values of wet body mass and abdomen length (Table 4). Hatchlings from different treatments differed in their scores on the second axis (One-way ANOVA; $F_{3, 68} = 4.36$, $P < 0.008$; Ft^{ab}, 27^b, 30^a, 33^{ab}) but not on the first axis (One-way ANOVA; $F_{3, 68} = 2.64$, $P = 0.056$).

Neither sprint speed nor the maximum distance was correlated with hatchling SVL within each thermal treatment (all $P > 0.10$). One-way ANOVA revealed that both sprint speed ($F_{3, 68} = 4.12$, $P < 0.01$) and the maximum distance ($F_{3, 68} = 4.45$, $P < 0.007$) differed among the four treatments, with hatchlings from the 27°C treatment performing much more poorly in the racetrack than did those from the other three treatments (Fig. 3). A partial correlation analysis examining the effects of the mean versus variance of incubation temperatures in the Ft treatment on locomotor performance of hatchlings revealed that: (1) sprint speed was negatively correlated with the thermal variance ($r = -0.30$, $t = 2.20$, $df = 16$, $P = 0.043$), but was positively correlated with the thermal mean ($r = 0.31$, $t = 2.38$, $df = 16$, $P = 0.030$); and

(2) neither the thermal variance ($r = 0.17$, $t = 1.21$, $df = 16$, $P = 0.246$) nor the thermal mean ($r = -0.18$, $t = 1.22$, $df = 16$, $P = 0.240$) affected the maximum length.

3 Discussion

Hatching success was high in the 30°C treatment (89%) but low in the other three treatments (44% – 76%), suggesting that hatchability is optimized at temperatures around 30°C in *L. reevesii*. However, what is the range of temperatures suitable for egg incubation in the species? Hatching success (44%) was lowest at 27°C, and hatchlings derived from eggs incubated at this temperature performed much more poorly in the racetrack than did hatchlings from the other three treatments (Fig. 3). Hatchlings from eggs incubated at 30°C and 33°C showed no important differences in the examined phenotypes, but hatching success decreased markedly at 33°C (74%). As the extent to which lizards enjoy reproductive benefits highly depends on how successfully their embryos can complete development, the temperatures that result in a marked decrease in hatching success or hatchling performance are certainly not suitable for embryonic development. Thus, although the temperatures of 27°C and 33°C support embryonic development in *L. reevesii*, these two temperatures are not ideal. Nest temperatures at the sites where we collected gravid females rarely varied outside the range of 28°C – 32°C, and exposure of eggs to temperatures outside this range for the whole (the 27°C and 33°C treatments) or some (the Ft treatment) incubation period had a detrimental effect on hatchability. These observations suggest that the range of temperatures suitable for incubation of *L. reevesii* eggs most probably lies between 28°C and 32°C.

Suitable incubation temperatures differ among reptiles from thermally different habitats or depositing eggs in thermally different nests. For example, eggs cannot be incubated at temperatures higher than 28°C in *S. modesta* (Lu et al., 2006) or higher than 25°C in the tuatara *Sphenodon punctatus* (Thompson, 1990) from cool habitats, whereas the detrimental effects on hatching success cannot be detected until eggs are incubated at temperatures higher than 30°C in lizards such as

Table 3 Size, mass and morphology of hatchlings from eggs incubated under different thermal regimes

	Thermal treatments				Statistical results
	Ft	27°C	30°C	33°C	
<i>n</i>	19	11	25	17	
Initial egg mass (g)	1.99 ± 0.11 (1.40 – 3.10)	1.92 ± 0.14 (1.55 – 2.99)	1.94 ± 0.06 (1.38 – 2.73)	2.06 ± 0.08 (1.58 – 2.78)	$F_{3, 68} = 0.40, P = 0.754$
Hatchling wet mass (g)	1.77 ± 0.08 (1.23 – 2.47)	1.59 ± 0.07 (1.34 – 2.09)	1.76 ± 0.06 (1.24 – 2.54)	1.73 ± 0.06 (1.32 – 2.35)	$F_{3, 67} = 2.54, P = 0.064$
Snout-vent length (mm)	39.3 ± 0.5 (34.9 – 44.2)	38.0 ± 0.5 (34.7 – 40.6)	38.6 ± 0.4 (35.6 – 43.6)	38.1 ± 0.5 (33.6 – 42.7)	$F_{3, 67} = 2.66, P = 0.055$
Abdomen length (mm)	17.6 ± 0.3 (15.8 – 20.2)	16.8 ± 0.3 (15.1 – 18.7)	17.5 ± 0.3 (15.5 – 21.6)	16.9 ± 0.3 (14.4 – 19.6)	$F_{3, 67} = 4.04, P = 0.011$ Ft ^a , 27 ^{ab} , 30 ^a , 33 ^b
Tail length (mm)	75.3 ± 1.2 (68.4 – 85.2)	70.5 ± 1.3 (65.8 – 78.1)	74.3 ± 1.3 (61.4 – 87.5)	73.0 ± 2.2 (57.1 – 85.6)	$F_{3, 67} = 1.75, P = 0.166$
Head length (mm)	10.0 ± 0.1 (9.4 – 11.0)	9.8 ± 0.1 (9.2 – 10.8)	9.9 ± 0.1 (9.3 – 10.7)	9.8 ± 0.1 (8.7 – 10.8)	$F_{3, 67} = 2.63, P = 0.057$
Head width (mm)	7.0 ± 0.1 (6.6 – 7.8)	6.9 ± 0.1 (6.3 – 7.6)	6.8 ± 0.1 (6.1 – 7.4)	6.7 ± 0.1 (6.0 – 7.4)	$F_{3, 67} = 5.65, P < 0.002$ Ft ^a , 27 ^{ab} , 30 ^b , 33 ^b
Fore-limb length (mm)	10.1 ± 0.1 (8.7 – 10.7)	9.9 ± 0.2 (9.0 – 11.3)	10.0 ± 0.1 (8.6 – 11.4)	9.8 ± 0.2 (8.0 – 10.9)	$F_{3, 67} = 0.87, P = 0.460$
Hind-limb length (mm)	18.2 ± 0.2 (16.4 – 20.2)	17.8 ± 0.3 (16.2 – 19.3)	17.8 ± 0.3 (15.6 – 20.9)	17.5 ± 0.3 (14.9 – 19.5)	$F_{3, 67} = 2.21, P = 0.095$

Data are expressed as Mean ± SE (range). *F* values of ANOVA (for initial egg mass) or ANCOVAs (for all the other hatchling variables, with initial egg mass as the covariate). Means corresponding to thermal treatments with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$, $a > b$).

Table 4 Loading of the first two axes of a principal component analysis on eight hatchling variables

	Factor loading	
	PC1	PC2
Wet body mass	0.337	0.784 *
Snout-vent length	0.648	0.668
Abdomen length	0.276	0.892 *
Tail length	0.644	0.336
Head length	0.839 *	0.392
Head width	0.853 *	0.247
Fore-limb length	0.786 *	0.269
Hind-limb length	0.800 *	0.416
Variance explained (%)	46.4	30.4

Size effects are removed in all cases by using residuals from the regressions on initial egg mass. * Variables with the main contribution to each factor.

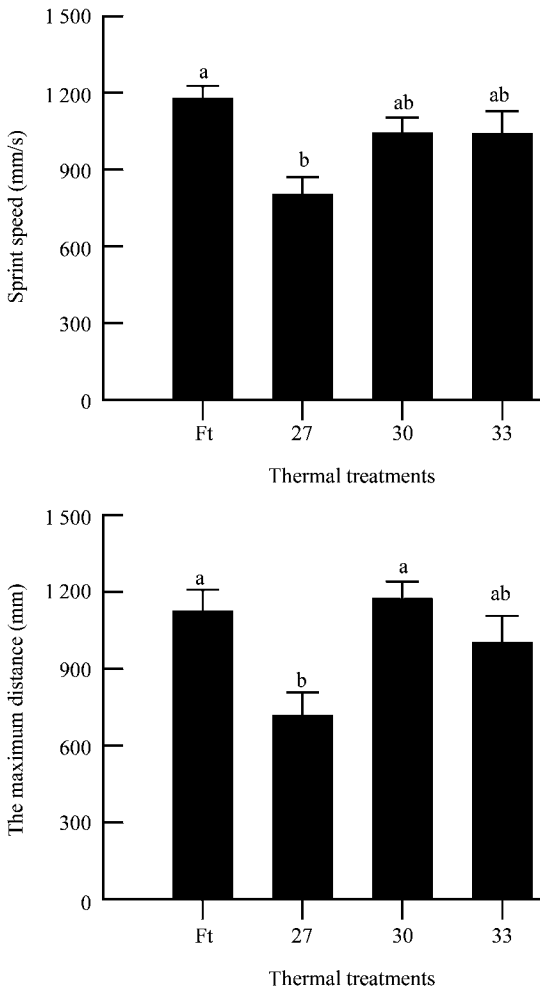


Fig.3 Mean values (\pm SE) for sprint speed and the maximum distance of hatchlings derived from eggs incubated under different thermal treatments regimes

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

T. septentrionalis (Lin and Ji, 1998), *E. chinensis* (Ji and Zhang, 2001), the northern African agamid lizard *Agama impalearis* (El Mouden et al., 2001) and the oriental garden lizard *Calotes versicolor* (Ji et al., 2002b) from warm habitats. Existing data show that, for most reptilian species, temperatures between 24°C and 31°C seem to be the most successful (Birchard, 2004). Compared with the result reported for *C. versicolor* in sympatry with *L. reevesii* in Ledong, the range of suitable incubation temperatures is relatively narrow in *L. reevesii*. As in *L. reevesii*, hatchability decreases noticeably at temperatures higher than 30°C in *C. versicolor* (Ji et al., 2002b). However, eggs can be incubated very successfully at 27°C in *C. versicolor* but not in *L. reevesii*, primarily because eggs of *C. versicolor* are deposited in shallow (and thus, thermally more variable) nests. Temperatures within natural nests selected by reproducing females of *L. reevesii* are characterized by the high mean by the small amplitude of thermal fluctuations. Our data therefore provide an inference that incubation temperatures are relatively high but narrow in lizards depositing eggs in warm and thermally stable nests.

In this study, hatchlings from eggs incubated under the three warmer thermal regimes did not differ from each other in locomotor performance, and these hatchlings on average performed much better in the racetrack than did hatchlings incubated at 27°C (Fig.3). Little variation was detected among measures of size and morphology of hatchlings across the four thermal treatments, although the differences in abdomen length and head width were statistically significant (Table 3). These observations indicate that locomotor (both sprint speed and the maximum length) rather than morphological phenotypes are more likely affected by incubation temperature in *L. reevesii*. Our results are consistent with the findings from similar studies of lizards that show the existence of a range of temperatures within which there are no detectable differential effects of incubation temperature on morphology of hatchlings (e.g., Braña and Ji, 2000; Ji and Zhang, 2001; Pan and Ji, 2001; Ji et al., 2002b; Hao et al., 2006).

Thermal fluctuations experienced during incubation positively affect locomotor performance of hatchlings in *T. septentrionalis* (Du and Ji, 2006), but not in the Mongolian racerunner *Eremias argus* (Hao et al., 2006). In *L. reevesii*, however, increased variance of incubation temperatures negatively affects locomotor performance (spring speed) of hatchlings. Unfortunately, whether these differences can be attributed to differential embryonic responses to thermal fluctuations among species or to extreme temperatures experienced by *L. reevesii* eggs in the Ft treatment is currently unknown.

Data from the Ft treatment show that the mean rather than the variance of incubation temperatures affects incubation length and that a substantial proportion

(~96%) of variation in incubation length can be explained by the thermal mean. Thus, as in the eastern fence lizard *Sceloporus undulatus* (Andrews et al., 2000) and the keelback snake *Tropidonophis mairii* (Webb et al., 2001), thermal fluctuations do not influence incubation length any differently than constant temperatures with the same mean in *L. reevesii*. In other squamate reptiles, however, thermal fluctuations may either increase (Hao et al., 2006) or reduce incubation length (Overall, 1994; Shine and Harlow, 1996). It seems that eggs of different species of squamate reptiles may respond differentially to thermal fluctuations.

Taken together, our data show that temperatures suitable for egg incubation are relatively high and narrow in *L. reevesii*. Locomotor rather than morphological phenotypes of hatchlings are more likely affected by incubation temperature in *L. reevesii*. Thermal fluctuations experienced during incubation negatively affect locomotor performance of hatchlings for some unknown reasons, but do not influence incubation length when holding the mean incubation temperature constant. Temperatures between 27°C and 33°C do not modulate the sexual phenotype of hatchlings, signifying that *L. reevesii* is a GSD (genetic sex determination) lizard.

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