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Does the variance of incubation temperatures always constitute a significant selective force for origin of reptilian viviparity?

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Abstract To test the hypothesis that the variance of incubation temperature may have constituted a significant selective force for reptilian viviparity, we incubated eggs of the slender forest skink *Scincella modesta* in five thermally different natural nests and at two constant temperatures (18 °C and 21 °C). Our manipulation of incubation temperature had significant effects on incubation length and several hatchling traits (snout-vent length, tail length, fore-limb length, and sprint speed), but not on hatching success and other hatchling traits examined (body mass, head size, and hind-limb length). Incubation length was nonlinearly sensitive to temperature, but it was not correlated with the thermal variance when holding the thermal mean constant. The 18 °C treatment not only produced smaller sized hatchlings but also resulted in decreased sprint speed. Eggs in the nest with the greatest proportion of temperatures higher than 28 °C also produced smaller sized hatchlings. None of the hatchling traits examined was affected by the thermal variance. Thermal fluctuations did result in longer incubation times, but females would benefit little from maintaining stable body temperatures or selecting thermally stable nests in terms of the reduced incubation length. Our data show that the mean rather than the variance of temperatures has a key role in influencing incubation length and hatchling phenotypes, and thus do not support the hypothesis tested [*Current Zoology* 58 (6): 812–819, 2012].

Keywords Viviparity, Scincid lizard, Developmental plasticity, Phenotype, Incubation length, Thermal

One distinction that has emerged in life-history theory is that of oviparous (egg-laying) versus viviparous (live-bearing) reproductive modes. Viviparity evolves from oviparity, the dominant reproductive mode among vertebrates, through gradual increases in the length of egg retention and intrauterine development until embryonic development is complete (Andrews and Mathies, 2000). Viviparity characterizes all mammals except monotremes, and it has also had more than 150 independent origins within non-mammalian vertebrates including fishes (Dulvy and Reynolds, 1997; Goodwin et al., 2002; Reynolds et al., 2002), amphibians (Duellman and Trueb, 1986; Wilkinson and Nussabaum, 1998; García-París et al., 2003), and squamate reptiles (Blackburn, 1982, 2000; Shine, 2005). Viviparity offers pervasive benefits by lowering embryonic mortality, accelerating developmental rate, optimizing the phenotype of offspring, freeing females to find suitable egg-laying sites, and providing the mother the chance to select the sex of her offspring in species with temperature-dependent sex determination, but entails several costs such as increased maternal mortality (Tinkle and Gibbons, 1977; Shine, 2005; Calderón Espinosa et al., 2006; Wapstra et al., 2009; Robert and Thompson, 2010).

The transition from oviparity to viviparity occurred in the past, presumably under conditions similar to those experienced by extant oviparous forms (Shine, 2002, 2004). Thus, with suitable animals, it is possible to infer processes at work in evolutionary history from present-day phenomena. Squamate reptiles (lizards, snakes, and amphisbaenians) provide unparalleled opportunities in this respect because: (1) nearly one-fifth of squamate reptiles are viviparous, and this reproductive mode has evolved far more frequently (> 100 separate lineages) in this group of animals than in all other non-mammalian vertebrates combined; and (2) unlike other reptilian taxa where females deposit when embryos are in the gastrula (turtles and tuataras) or the neurula (crocodilians) stage, squamate reptiles exhibit nearly the entire gamut of

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possible embryonic stages at oviposition, with embryonic stages at oviposition grouping around a mode of Dufaure and Hubert's (1961) stage 30 (lizards) or Zehr's (1962) stage 27 (snakes) in most species (Shine, 1983, 1995, 2002, 2005; Xavier and Gavaud, 1986; Andrews and Mathies, 2000). Many hypotheses have been proposed since at least the 1930s on the selective forces leading to the evolution of viviparity within squamate reptiles, with most of them postulating that viviparity has evolved 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 (Shine, 1995, 2004, 2005; Blackburn, 2000, 2006). However, whether the thermal variance (a measure of thermal variability), the thermal mean, or both play an important role in the selective forces for vivipaity still remains poorly known due to the paucity of studies examining the ways that specific attributes of incubation thermal regimes affect hatchling phenotypes.

Studies of reptiles designed to test the hypothesis that not only the thermal mean but also the way in which incubation temperatures fluctuate around given mean levels can affect embryos and hatchlings have not yet reached any consistent conclusion regarding the influence of thermal variability on hatchling traits. For example, fluctuating temperatures influence hatchling phenotypes differently than constant temperatures with the same mean temperature in some species (Shine et al., 1997; Ashmore and Janzen, 2003; Shine, 2004; Mullins and Janzen, 2006; Les et al., 2007), but not in others (Demuth, 2001; Chen et al., 2003; Hao et al., 2006; Lin et al., 2008; Lu et al., 2009). As the effects demonstrated in fluctuating-temperature incubation could be due to the fact that thermal fluctuations result in exposure of embryos to deleterious extreme temperatures (Lu et al., 2009; Li et al., 2012), this inconsistency raises a question that forms the basis of this study: Does the thermal variance *per se* plays a key role in shaping the phenotype of hatchlings, and thus constitute a significant selective force for viviparity? If so, we may hypothesize that the phenotype of hatchlings should be sensitive to the thermal variance even if developing embryos are never exposed to extreme temperatures.

Here, we describe a study incubating eggs of the slender forest skink *Scincella modesta* in five natural nests and at two constant temperatures (see below for details) to test our hypothesis. This small sized (up to 55 mm snout-vent length, SVL), oviparous skink is distributed in the eastern and central provinces of China, and shows a preference for shaded forest habitats where ambient temperatures are rarely higher than 30 °C even in the hottest months (Huang, 1999; Lu et al., 2006). Females lay a single clutch of eggs per breeding season between May and June in shallow nests where temperatures vary appreciably in response to oscillations in air temperature (Lu et al., 2006). The modal embryonic stage at oviposition is Stage 31 in the Dufaure and Hubert's (1961) developmental series (Li, 2009[1]).

1 Materials and Methods

1.1 Animal collection and egg incubation

We collected 11 gravid females (42–50 mm SVL) in mid-May 2011 from a population in Hangzhou (30°16′N, 120°12′E), Zhejiang, East China. Females were brought to our laboratory in Hangzhou, approximately 8 km away from the site where they were collected. Females were individually housed in 20 cm × 15 cm × 20 cm (length × width × height) glass cages with 5 cm depth moist soil, small pieces of clay tiles, grasses, and a 20-W spotlight mounted in each cage to allow thermoregulation during daylight hours (06:00–19:00 h; Beijing time). These cages were placed in a room where temperatures were controlled within the range of 18–24 °C. Females were fed a combination of mealworms (larvae of *Tenebrio molitor*), house crickets *Achetus domesticus* and field-captured grasshoppers, and water enriched with vitamins and minerals was provided *ad libitum*.

The cages were checked at least thrice daily as soon as the first females laid eggs, such that eggs were always collected, measured for length and width and weighed within a few hours after being laid. The viability of freshly laid eggs was judged by the presence of an embryonic disc using a spotlight. Post-oviposition females were measured and weighed, and were then released at their point of capture.

Females laid a single clutch of 5–10 eggs between 22 May and 6 June. Of the 88 eggs collected, three eggs laid by two females were not included in the experiment because they were infertile. The remaining 85 eggs were individually placed in covered plastic jars (20 ml) with known amounts of vermiculite and water at about –12

[1] Li H, 2009. The evolution of reptilian viviparity and its adaptive significance using lizards as the model systems. Ph.D. dissertation, Nanjing Normal University, Nanjing, Jiangsu, China.

kPa water potential (1 g dried vermiculite: 2 g water; Lu et al., 2006). A half of the egg was buried lengthwise in the substrate, with the surface near the embryo exposed to air inside the jar. Twenty-five eggs were incubated at two constant temperatures of 18.0 ± 0.5 °C and 21.0 ± 0.5 °C (hereafter the 18 and 21 °C treatments, respectively) using two Shellab incubators (Sheldon MFG Inc, USA), and the remaining 60 eggs were assigned to one of the five natural nests (hereafter the N1, N2, N3, N4 and N5 treatments, respectively) located in the field. Eggs from single clutches were divided among the seven temperature treatments as equally as possible. One TK-0014 Tinytalk datalogger (Gemini Pty, Australia) programmed to record temperature at 1-hour intervals was placed in the center of each nest throughout the incubation period, so that thermal environments experienced by each egg could be tracked.

1.2 Measurement of hatchling phenotypes

Incubation length, measured as the time between oviposition and pipping, was recorded for each egg. A total of 68 eggs hatched. Newly emerged hatchlings were weighed and then used to evaluate the effects of incubation temperature on sprint speed and morphological traits. We conducted all locomotor trials at the body temperature of 28 °C, which was achieved by placing hatchlings into a Shellab incubator at the correspondent temperature for a minimum of 30-min prior to testing. Hatchlings were individually chased down the length of a 1.6 m racetrack with one transparent side through which they were filmed with a Panasonic NV-DS77 digital video camera (Panasonic, Japan). Each hatchling was run twice, with a minimum of 30 min rest between the two trials. The tapes were later examined on a frame-by-frame basis using MGI VideoWave III software (MGI Software Co., Canada) for sprint speed in the fastest 25-cm interval.

Immediately following locomotor trials, we cooled hatchlings to about 5 °C by placing them on a woody cooling box, and then measured them (to the nearest 0.01 mm) with Mitutoyo digital calipers (Kanagawa, Japan). Morphological measurements taken for each hatchling 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 length (humerus plus ulna), and hind-limb length (femur plus tibia). All hatchlings were released to the field following the collection of morphological data.

1.3 Statistical analyses

Statistical analyses were performed with STATISTICA software (version 6.0 for PC). We used *G*-test, Kruskal-Wallis test, linear regression analysis, partial correlation analysis, one-way analysis of variance (ANOVA), one-way analysis of covariance (ANCOVA), multivariate analysis of covariance (MANCOVA), Tukey's *post hoc* test, and principal component analysis to analyze the corresponding data. Prior to parametric analyses, we tested data for normality using the Kolmogorov-Smirnov test, and for homogeneity of variances using the Bartlett's test (univariate level) or the Box's M test (multivariate level). The homogeneity of slopes was checked prior to examining differences in the adjusted means. Throughout this paper, values are presented as mean ± standard error (SE), and the significance level is set at $\alpha = 0.05$.

2 Results

2.1 Hatching success and incubation length

Table 1 reports values for the thermal environments experienced by the eggs in the five natural nests. Both the mean (Kruskal-Wallis test, $H_{4,\ N = 46} = 43.00$, $P < 0.0001$) and the variance (the standard deviation squared) (Kruskal-Wallis test, $H_{4,\ N = 46} = 31.45$, $P < 0.0001$) of temperatures differed among eggs incubated in the five nests, and there was a positive relationship between the thermal variance and the thermal mean ($F_{1,\ 44} = 129.57$, $P < 0.0001$; Fig. 1).

Table 1 The mean, minimum, maximum and variance of temperatures (°C) experienced by *S. modesta* eggs hatched in the five natural nests

	Mean	Minimum	Maximum	Variance	% of temperatures higher than 28 °C
Nest 1	21.8 ± 0.03 (21.7–21.9)	18.4	26.3 ± 0.3 (25.5–27.0)	4.35 ± 0.01 (4.31–4.40)	0
Nest 2	22.4 ± 0.06 (22.1–22.5)	18.4	26.9 ± 0.4 (25.5–28.8)	4.59 ± 0.25 (4.00–5.90)	0.5 ± 0.4 (0–2.4)
Nest 3	22.7 ± 0.04 (22.5–22.9)	18.4 ± 0.03 (18.1–18.4)	27.8 ± 0.3 (26.3–29.2)	5.28 ± 0.32 (4.14–6.54)	1.2 ± 0.5 (0–3.6)
Nest 4	23.3 ± 0.05 (23.1–23.5)	18.4 ± 0.1 (18.1–18.8)	28.9 ± 0.1 (28.4–29.2)	6.38 ± 0.20 (5.01–6.98)	3.1 ± 0.4 (0.1–4.3)
Nest 5	23.8 ± 0.1 (23.5–24.9)	18.7 ± 0.2 (18.1–20.2)	28.9 ± 0.2 (27.4–29.6)	7.06 ± 0.20 (6.31–8.62)	3.6 ± 0.6 (0–7.8)

Values are expressed as mean ± SE (range). Six, 9, 10, 9 and 12 eggs hatched in the N1, N2, N3, N4 and N5 treatments, respectively.

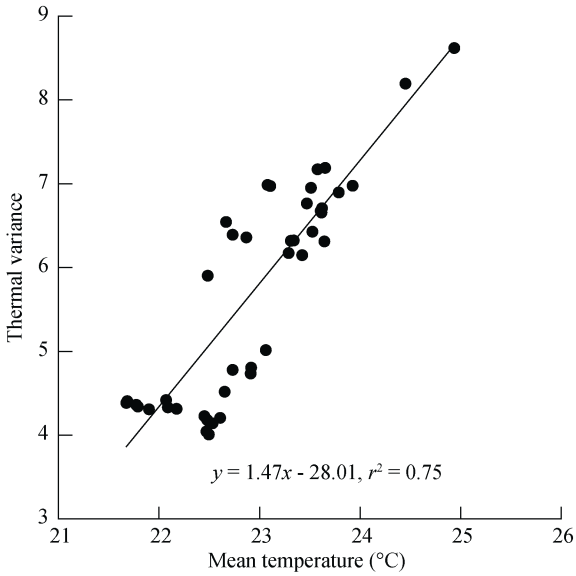

Fig. 1 The relationship between the thermal variance and the mean of ambient temperatures experienced by eggs incubated in the five natural nests

The regression equation and coefficient are indicated in the figure

Table 2 Effects of incubation thermal environments on incubation length and hatching success. Data on incubation length are expressed as mean ± SE (range)

Thermal treatments	Incubated eggs	Incubation length (d)	Hatching success (%)
18 °C	13	65.1 ± 0.8 (60.5–69.0)	92.3 (12/13)
21 °C	12	36.2 ± 0.5 (33.3–38.1)	83.3 (10/12)
Nest 1	7	36.0 ± 0.5 (34.0–37.0)	85.7 (6/7)
Nest 2	13	31.4 ± 0.6 (29.0–33.2)	69.2 (9/13)
Nest 3	13	31.0 ± 0.5 (28.6–33.2)	76.9 (10/13)
Nest 4	13	28.2 ± 0.6 (26.0–32.0)	69.2 (9/13)
Nest 5	14	27.3 ± 0.4 (25.3–29.1)	85.7 (12/14)

Eggs incubated under the seven thermal regimes did not differ from each other in mean mass at oviposition (one-way ANOVA, $F_{6, 61} = 0.58$, $P = 0.748$). Hatching success did not differ among the seven temperature treatments ($G = 0.42$, $df = 6$, $P > 0.95$; Table 2). Incubation length differed among the seven treatments (one-way ANOVA, $F_{6, 61} = 561.99$, $P < 0.0001$), and was nonlinearly sensitive to temperature (Fig. 2). We found a negative relationship between incubation length and the thermal mean in the eggs incubated in nature ($F_{1, 44} = 41.12$, $P < 0.0001$). A partial correlation analysis revealed that incubation length was not correlated with the thermal variance when holding the thermal mean constant ($r = 0.16$, $t = 1.04$, $df = 43$, $P = 0.303$). Embryos developed more slowly at fluctuating temperatures than at equivalent constant temperatures (one-way

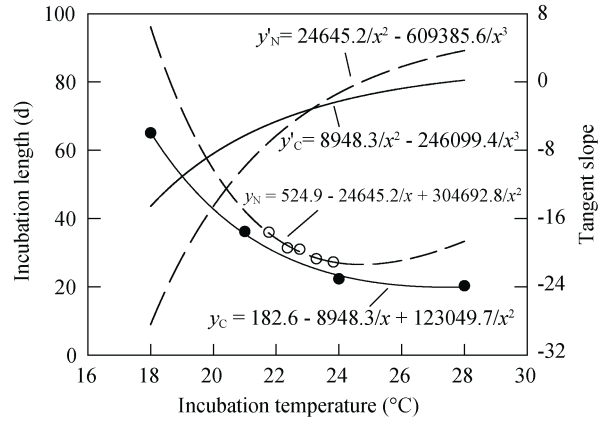

Fig. 2 The curvilinear regressions of incubation length on incubation temperature

Data supporting the dots of 24 °C and 28 °C are from an earlier study of *S. modesta* (Lu et al., 2006). Solid dots and curves represent eggs incubated at constant temperatures, and open dots and dash lines represent eggs incubated in natural nests. The functions for the curvilinear regressions and the derived functions for instantaneous variation in tangent slopes are given in the figure

ANCOVA, $F_{1, 7} = 60.71$, $P < 0.0002$), with the expected incubation lengths being on average increased by 3.6 days in the five nests.

2.2 Hatching phenotypes

The variance of temperatures experienced by eggs during incubation in the five nests was not a significant predictor of all examined hatchling traits (simple linear regression, all $P > 0.060$). Within each treatment, egg mass was not a significant predictor of sprint speed (simple linear regression, all $P > 0.183$), but explained a substantial amount of variation in the examined morphological phenotypes (simple linear regression, all $P < 0.005$).

Sprint speed differed among the seven treatments ($F_{6, 61} = 6.27$, $P < 0.0001$), and it was noticeably lower in the 18 °C treatment than in the other six treatments (Fig. 3). Morphological traits overall differed among hatchlings incubated under the seven temperature regimes (MANCOVA with egg mass as the covariate, Wilks' lambda = 0.075, $df = 42, 256$, $P < 0.0001$; Table 3). Specifically, SVL was significantly greater in the N1 treatment than in the 18 °C and N5 treatments (Tukey's *post hoc* test, both $P < 0.034$), tail length was significantly greater in the 21 °C and N1-3 treatments than in the 18 °C treatment (Tukey's *post hoc* test, all $P < 0.007$), and fore-limb length was significantly greater in the 18 °C and N1 treatments than in the N5 treatment (Tukey's *post hoc* test, both $P < 0.027$). The remaining morphological traits, including body mass, head length, head width and hind-limb length, did not differ signifi-

cantly among the seven treatments (Tukey's *post hoc* test, all $P > 0.091$).

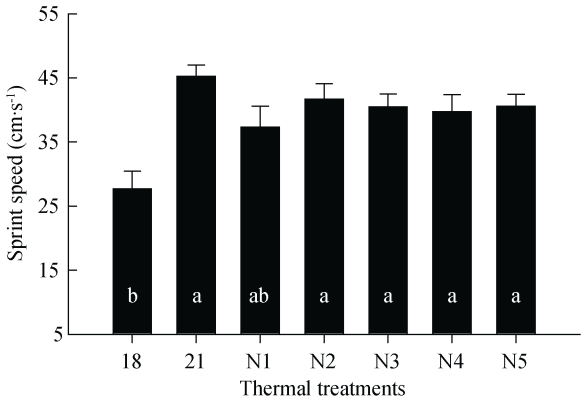

Fig. 3 Means (+SE) for sprint speed of hatchlings from different temperature treatments

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

A principal component analysis resolved two components (with eigenvalues ⩾ 1) from the seven morphological variables, accounting for 65.6% of the variation in the original data (Table 4). The first component (46.3% variance explained) had high positive loading for egg size-free values of SVL, tail length, fore-limb length, and hind-limb length, and the second component (19.3% variance explained) had high negative loading for the egg size-free value of head length (Table 4). Hatchlings from the seven treatments differed in their scores on the first (one-way ANOVA, $F_{6, 61} = 2.54$, $P = 0.029$) and second axes (one-way ANOVA, $F_{6, 61} = 5.43$, $P < 0.0002$), and could be divided into three groups respectively including hatchlings from the 18 °C treatment, from the 21 °C and N1-4 treatments, and from the N5 treatment (Fig. 4).

Table 3 Size and morphology of hatchlings incubated under the seven thermal regimes. Data are expressed as mean ± SE (range)

	Thermal treatments						
	18 °C	21 °C	Nest 1	Nest 2	Nest 3	Nest 4	Nest 5
N	12	10	6	9	10	9	12
Egg mass (mg)	87.2 ± 3.2 (62.3–106.7)	90.1 ± 2.5 (77.5–103.2)	88.9 ± 2.5 (82.7–97.9)	93.0 ± 2.6 (82.7–102.8)	87.5 ± 3.9 (63.0–102.6)	88.8 ± 4.5 (62.0–103.4)	85.2 ± 2.9 (62.6–100.7)
Snout-vent length (mm)	16.5 ± 0.2 (15.2–17.3)	17.4 ± 0.2 (16.0–18.2)	17.7 ± 0.2 (17.3–18.5)	17.5 ± 0.2 (16.6–18.5)	17.3 ± 0.3 (15.7–18.1)	17.2 ± 0.3 (15.8–18.2)	16.5 ± 0.3 (14.1–18.0)
Tail length (mm)	17.5 ± 0.4 (13.7–18.8)	21.4 ± 0.5 (17.6–23.7)	21.2 ± 0.4 (20.4–22.5)	20.7 ± 0.4 (19.4–22.8)	20.3 ± 0.6 (16.9–22.7)	19.6 ± 1.0 (14.0–22.5)	18.9 ± 0.7 (13.6–22.0)
Body mass (mg)	95.5 ± 4.1 (65.5–112.3)	101.1 ± 4.1 (77.3–117.6)	105.7 ± 3.1 (98.5–117.1)	103.6 ± 4.3 (82.7–116.8)	101.2 ± 5.0 (71.8–122.5)	102.8 ± 4.3 (80.3–114.2)	97.6 ± 4.6 (68.5–118.4)
Head length (mm)	4.3 ± 0.06 (3.9–4.6)	4.2 ± 0.05 (4.0–4.5)	4.3 ± 0.1 (4.0–4.6)	4.3 ± 0.04 (4.1–4.6)	4.1 ± 0.06 (3.8–4.4)	4.3 ± 0.1 (3.7–4.6)	4.1 ± 0.08 (3.7–4.7)
Head width (mm)	2.7 ± 0.04 (2.4–2.8)	3.1 ± 0.02 (3.0–3.2)	2.7 ± 0.04 (2.6–2.9)	3.0 ± 0.03 (2.8–3.1)	2.7 ± 0.04 (2.5–2.9)	2.6 ± 0.07 (2.2–3.1)	2.6 ± 0.05 (2.3–3.0)
Fore-limb length (mm)	3.4 ± 0.1 (2.8–3.6)	3.2 ± 0.06 (2.9–3.4)	3.4 ± 0.1 (3.2–3.6)	3.4 ± 0.05 (3.1–3.5)	3.3 ± 0.1 (2.7–3.6)	3.3 ± 0.1 (2.8–3.7)	3.0 ± 0.1 (2.5–3.4)
Hind-limb length (mm)	3.9 ± 0.1 (3.2–4.2)	4.0 ± 0.06 (3.7–4.3)	3.9 ± 0.1 (3.8–4.2)	3.9 ± 0.04 (3.7–4.1)	3.9 ± 0.1 (3.1–4.4)	4.0 ± 0.1 (3.1–4.5)	3.7 ± 0.1 (3.0–4.1)

3 Discussion

Our manipulation of incubation thermal environment had significant effects on incubation length and several hatchling traits (SVL, tail length, fore-limb length, and sprint speed), but not on hatching success and other hatchling traits examined (body mass, head length, head width, and hind-limb length). Results of this study, together with those of an earlier study incubating *S. modesta* eggs at three constant temperatures (24, 28, and 30 °C; Lu et al., 2006), allow us to discuss whether the variance of incubation temperatures has a key role in the selective force for viviparity in reptiles.

An earlier study of *S. modesta* shows that: (1) the temperature of 30 °C is lethal to embryos, whereas hatching successes at 24 °C (~97%) and 28 °C (~95%) are both impressively high; and (2) hatchlings incubated at 28 °C run much more slowly than do their counterparts incubated at 24 °C, although they do not differ in any examined morphological trait (Lu et al., 2006). What can be inferred from these results is that the temperature of 28 °C is suboptimal for incubation of *S. modesta* eggs. In the present study, we further find that

Table 4 Loading of the first two axes of a principal component analysis on seven hatchling morphological traits

	Factor loading	
	PC1	PC2
Snout-vent length	**0.870**	0.297
Tail length	**0.725**	0.507
Wet body mass	0.631	0.395
Head length	0.445	**–0.721**
Head width	0.498	0.085
Fore-limb length	**0.714**	–0.437
Hind-limb length	**0.779**	–0.364
Proportion of variance explained (%)	46.3	19.3

Size effects are removed in all cases by using residuals from the regressions on egg mass at oviposition. Morphological traits with the main contribution to each factor in bold face font

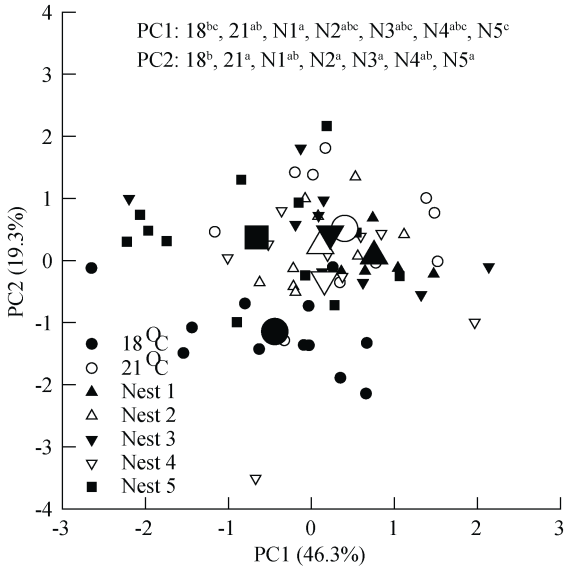

Fig. 4 Positions of *S. modesta* hatchlings incubated under the seven thermal regimes (symbols on the bottom left corner) in the space defined by the first two axes of a principal component analysis (eigenvalues ⩾ 1) based on seven morphological variables

Effects of egg size were removed using residuals from the regressions of corresponding variables on egg mass at oviposition. Larger symbols represent the mean scores on the two axes. Means with different superscripts differ significantly (Tukey's *post hoc* test, $\alpha = 0.05$, a > b > c)

the lower threshold of temperatures optimal for incubation of *S. modesta* eggs should be higher than 18 °C, because the 18 °C treatment not only produced smaller-sized hatchlings but also resulted in decreased running speed (Table 3, Fig. 3). Incubation length decreased by approximately 29 days as temperature increased from 18 °C to 21 °C (Table 2), and the 21 °C treatment produced larger-sized hatchlings that performed very well in the racetrack (Fig. 3). These observations provide compelling evidence that the temperature of 21 °C falls within the range of temperatures optimal for incubation of *S. modesta* eggs.

Mean incubation temperatures (21.8–23.8 °C) in the five nests were all within the range of temperatures optimal for incubation of *S. modesta* eggs. So, why did hatchlings from the N5 treatment morphologically differ from their counterparts from the N1-4 treatments? The answer probably lies in that, as in other reptiles (Lu et al., 2009 and references therein), prolonged exposure of *S. modesta* eggs to extreme temperatures can substantially modify hatchling morphological phenotypes. Mean values for the thermal variance and the proportion of incubation temperatures higher than 28 °C were both greatest in the N5 treatment (Table 1). However, as in the N1-4 treatments, the thermal variance was not a significant predictor of all examined hatchling phenotypes in the N5 treatment. Hatchlings from the N5 treatment had significantly smaller body sizes as compared with those from the N1 treatment where eggs were never exposed to temperatures higher than 27 °C (Table 1). This difference is highly consistent with studies of many other reptiles where eggs incubated at high temperatures produce hatchlings with shorter body lengths as compared with those incubated at moderate temperatures (Deeming, 2004 and references therein), but does not provide any evidence for the importance of the thermal variance in determining this hatchling trait. Mean values for the proportion of incubation temperatures higher than 28 °C in the N4, N3 and N2 treatments were 3.1%, 1.2%, and 0.5%, respectively. Interestingly, however, hatchlings from these three treatments did not differ from their counterparts from the N1 treatment in any examined trait, presumably because brief periods of daily exposure of *S. modesta* eggs to temperatures suboptimal for embryonic development do not necessarily modify hatchling phenotypes.

One widespread phenomenon among reptiles is that incubation length increases at an ever-increasing rate as temperature decreases within the range where successful embryonic development can take place (Birchard, 2004). This pattern of the relationship between developmental rate and incubation temperature provides an inference that eggs incubated at fluctuating temperatures should take a longer time to hatch than those at constant temperatures with the same mean. In *S. modesta*, incubation length decreases by approximately 29 days as temperature increases from 18 °C to 21 °C, 14 day from 21 °C

to 24 °C and 2 days from 24 °C to 28 °C (Table 2; Lu et al., 2006), thus showing a pattern similar to the aforementioned one. Consistent with what was expected *S. modesta* embryos developed more slowly at fluctuating temperatures than at equivalent constant temperatures (Fig. 2). However, as incubation length was not correlated with the thermal variance when holding the thermal mean constant, the thermal variance *per se* is not a determinant of this thermally sensitive trait.

Mean incubation lengths overall increased by 3.6 days within the range of the fluctuating temperatures in the five nests. This suggests that females of *S. modesta* in nature would benefit little from maintaining stable body temperatures (during the phase between ovulation and laying) or selecting thermally stable nests (during the phase between oviposition and hatching) in terms of the reduced incubation length. It is worth noting that reptiles as ectotherms cannot provide stable thermal environments for their developing embryos without a concomitant increase in reproductive costs associated with more precise thermoregulation and/or nest-selection, and this is particularly true for species living in thermally challenging environments. Thus, most probably, the insensitivity of embryogenesis to the variance of incubation temperatures is a mechanism evolving in *S. modesta* that allows females to maximize their reproductive benefits at relatively low costs. A similar conclusion has also been drawn in other squamate reptiles such as the Mongolian racerunners *Eremias argus* (Hao et al., 2006), the many-lined sun skink *Mabuya multifasciata* (Ji et al., 2007), the Chinese cobra *Naja atra* (Lin et al., 2008) and the checkered keelback snake *Xenochrophis piscator* (Lu et al., 2009) where the thermal variance has no direct role in shaping the phenotype of hatchlings. In *M. multifasciata*, a warm-climate viviparous skink, gravid females do regulate body temperature more precisely and, thus, maintain more stable body temperatures than do nongravid females and adult males, not because stable maternal temperatures result in the optimization of offspring phenotypes but because the range (29–32 °C) of temperatures optimal for embryonic development is narrow in the skink (Ji et al., 2007).

When setting the tangent slope of the function depicting instantaneous variation in developmental rate at zero, we find that incubation length is theoretically minimized at 24.7 °C for eggs incubated in nature, and at 27.5 °C for eggs incubated at constant temperatures (Fig. 2). Presumably because temperatures that maximize the rate of embryonic development, or minimize incubation length do not maximize hatching success and hatchling phenotypes (Birchard, 2004; Deeming, 2004; Booth, 2006), in no nest located in the field was the overall mean incubation temperature higher than 24.7 °C (Table 1). This finding together with the observation that in no nest were eggs exposed to temperatures lower than 18 °C suggest that females of *S. modesta* always try to find thermally suitable egg-laying sites where successful embryonic development can take place.

In summary, our data show that the mean rather than the variance of incubation temperatures has a key role in influencing incubation length and hatchling phenotypes in *S. modesta*, and that incubation temperatures fluctuating within some thresholds do not have any important effects on hatchling phenotypes. *Scincella modesta* is among reptilian species where thermal fluctuations during incubation have no direct role in modifying hatchling traits as long as eggs are not exposed to extreme temperatures for long periods of time. Data gathered in *S. modesta* do not support the idea that stable temperatures should favour the evolution of viviparity (Shine, 2004; Webb et al., 2006), and show that the variance of incubation temperatures does not always constitute a significant selective force for reptilian viviparity.

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