Fluctuations in incubation temperature affect incubation duration but not morphology, locomotion and growth of hatchlings in the sand lizard Lacerta agilis (Lacertidae)

LI Hong, ZHOU Zongshi, DING Guohua, JI Xiang
(College of Life Sciences, Nanjing Normal University, NanJing 210046)

Abstract: Studies looking for potential effects of temperature and temperature fluctuations on phenotypic traits of reptile hatchlings have shown species variation, but have not always allowed a distinction between effects of fluctuation per se and temperature extremes themselves. To examine whether incubation temperature fluctuation has a key role in influencing the phenotype of offspring, we incubated eggs of the sand lizard Lacerta agilis at one of the four temperature regimes (27℃, 27±2℃, 27±4℃ and 27±6℃). We found that: (1) hatchlings incubated under the four temperature regimes did not differ from each other in any of morphological and physiological traits examined; (2) interactions that included temperature treatment did not affect any trait examined; (3) the mean incubation length was longer in the 27±6℃ treatment than in the other three treatments; and (4) female hatchlings were shorter in head length and width but longer in SVL as well as abdomen length than males derived from the same sized egg. Our data show that both the type and the magnitude of temperature variation can affect incubation length. We found no evidence for phenotypic divergence in responses to temperature fluctuations during incubation, and therefore suggest that temperature variation does not affect the phenotype of hatchlings in L. agilis.

Keywords: Lacertidae; Lacerta agilis; thermal fluctuations; incubation temperature; phenotype of hatchlings; incubation length; growth

0 Introduction

Phenotypic adjustment to variation in environmental conditions is widespread among organisms, and the processes that promote phenotypic plasticity are critical to understanding how they adapt to their environment (Kingsolver and Huey 1998). Among environmental factors likely inducing phenotypic variation, temperature is the single most important factor affecting many biological processes in organisms. The effects of temperature on developing embryos are more pronounced than those observed in later ontogenetic stages (Johnston et al., 1996; Lindström, 1999; Birchard, 2004). In reptiles, developmental temperature affects not only the rate of embryogenesis (measured as total incubation length), but also numerous important traits of offspring, including body size and shape, locomotor performance, behavior, growth, immune response, and even sexual differentiation in species with temperature-dependent sex determination (TSD) (Deeming 2004; Rhen and Lang 2004; Valenzuela 2004; Booth 2006; Les et al. 2009). According to the maternal manipulation hypothesis, maternal reptiles can manipulate the phenotype of their offspring to attain high fitness by selecting (through nest selection) or maintaining (through thermoregulation) appropriate temperatures for developing embryos (Shine 1995, 2005).

Most studies that assess the temperature effects on embryogenesis in reptiles have been based on oviparous species where eggs are incubated at constant temperatures, although

temperatures within natural nests often are far from constant. Incubation of reptile eggs at constant temperatures does allow examination of developmental rate, hatching success and offspring phenotype at any given temperature, but the temperature effects demonstrated in such an

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Foundations: National Science Foundation of China (No.30670281) and Chinese Ministry of Education (No.20103207120009)

Brief author introduction: Li Hong, (1982-), Male, Lecturer, Doing research in herpetology.
Correspondance author: Ji Xiang, (1963-), Male, Professor, Doing research in herpetology. E-mail: xji@mail.hz.zj.cn

experimental approach do not reflect what occurs in nature (Overall 1994; Ackerman and Lott 2004; Birchard 2004; Booth 2006). For example, eggs of *Eumeces chinensis* (Chinese skink; Ji and Zhang 2001; Chen et al. 2003) and *Naja atra* (Chinese cobra; Ji and Du 2001; Lin et al. 2008) cannot be incubated successfully at constant temperatures lower than 24 °C or higher than 32 °C, but exposure of eggs to temperatures outside the range of 24-32 °C has no adverse effects on hatching success and hatchling phenotype in the two species. Previous studies generally show that development can proceed successfully in nature across a much broader range of temperatures than that expected in constant-temperature incubation provided that exposure of eggs to extreme temperatures is intermittent (Overall 1994; Birchard 2004).

Studies of incubating reptile eggs at temperatures fluctuating sinusoidally or under temperature regimes mimicking natural conditions have shown that thermal fluctuations affect the phenotype of offspring in some species, but not in others (Lu et al. 2009 and references therein). This inconsistency, though likely reflecting to some extent the differences in the temperature protocols used, the differences in embryonic thermal tolerance among species, and/or the confounding influence of maternal and genetic factors on phenotypic expression, raises a question of whether incubation temperature fluctuation has a key role in influencing the phenotype of offspring. In species where fluctuating temperatures affect the phenotype of offspring differently than constant temperatures, that effect could be due to either the thermal fluctuation per se, or the fact that thermal fluctuations may result in exposure of eggs to extreme temperatures that increase embryonic mortality and/or result in major phenotypic modifications (Lin et al. 2008; Lu et al. 2009). Thus, to answer the above question, we need to examine whether and how temperature fluctuations affect the phenotype of offspring.

In this study, we evaluate phenotypic responses of hatchling sand lizards (*Lacerta agilis*) to constant versus fluctuating incubation temperatures using a CTE (constant temperature equivalent) design to hold the thermal mean constant (see below for details). We chose this cold-climate lizard for study because it has a wider range (perhaps wider than 12 °C; Jensen 1981) of temperatures at which successful embryonic development can take place than many temperate or warm-climate squamate reptiles (often narrower than 8 °C; Lu et al. 2006, 2009; Lin et al. 2008, 2010; Andrewartha et al. 2010), therefore allowing a determination of the temperature effects across a greater magnitude of variation in incubation temperature.

1 Materials and methods

1.1 Study species and husbandry

The sand lizard is a ground-dwelling oviparous lacertid lizard with a distribution across most of Europe and eastwards to Mongolia and Xinjiang of China (Bischoff 1988; Zhao 1999). The geographic range of this species extends further north than does that of any other oviparous lizard in the Eurasian continent (Zhao 1999; Arnold and Burton 2004). Many aspects of its ecology and biology have already been studied intensively but, surprisingly, only a handful of studies provide some information on its egg incubation (Jensen 1981; Olsson et al. 1996; Olsson and Shine 1997; Zhao 1999).

We collected 21 gravid females (80.4-99.5 mm snout-vent length, SVL) between 1-15 May 2008 from Yining (43°93’N, 81°48’E; ~1140 m elevation), Xinjiang, China. Females were transported to our laboratory in Nanjing, where they were housed in a 2.5 m x 1.2 m x 0.6 m (length x width x height) cage, in an indoor animal holding facility. The cage contained a substrate of sand (~50 mm depth), with grasses and pieces of clay tiles provided as cover. Thermoregulatory opportunities were provided during daytime hours by two 100-W incandescent lamps; overnight temperatures followed indoor ambient temperatures (21-28℃). Mealworm larvae (*Tenebrio molitor*), house crickets (*Achetus domestica*) and field-captured grasshoppers (Catantops spp.) dusted with multivitamins and minerals were provided daily, so that excess food was always available in the cage. Fresh water was also provided daily. Females with shelled oviductal eggs were housed individually in 40 cm x 30 cm x 25 cm egg-laying cages with moist sand, and a 20-W spotlight mounted in each cage to allow thermoregulation. Females laid a single clutch of 3-10 eggs between late May and late June.

1.2 Egg collection and incubation

Eggs were collected and weighed less than 3 h post-laying, to minimize water uptake or loss between the egg and the substrate. The viability or fertility of freshly laid eggs was identified by the presence of an embryonic disc using a spotlight. Post-oviposition females were measured for SVL and weighed. Of the 159 eggs collected, 122 could be incubated. These eggs were individually placed in covered plastic jars (50 ml) with known amounts of vermiculite and water at about −12 kPa water potential (2 g dried vermiculite: 1 g water; Lin and Ji 1998). Half of the egg was buried lengthwise in the substrate, with the surface near the embryo exposed to air inside the jar. Eggs from the same clutch were assigned as equally as possible among the four preprogrammed Binder KB incubators (Binder, Germany): one kept at a constant 27 °C; the other three kept at 27 ± 2 °C, 27 ± 4 °C and 27 ± 6 °C, respectively. Thermal fluctuations were maintained at 12 h (+) and 12 h (–), and were confirmed with Tinytalk temperature loggers (Gemini Pty, Australia) programmed to record temperature once every 30 min on 12 consecutive days (Fig. 1). We rotated jars every other day to compensate for possible undetected thermal gradients within the incubator. Water potential was adjusted every 5 days by individually weighing jars, and water was added as necessary to compensate for evaporative losses and water taken up by eggs.

1.3 Measurement of hatchling phenotypes

A total of 91 eggs hatched. Incubation length was defined as the time between oviposition and pipping. Upon emergence, hatchlings were collected, weighed, and measured firstly for locomotor performance and then for morphological traits. We conducted all locomotor trials at the body temperature of 30 °C, which was achieved by placing the hatchlings in a Shellab incubator (Sheldon MFG Inc., USA) at 30 °C for 30 min prior to testing. Locomotor performance

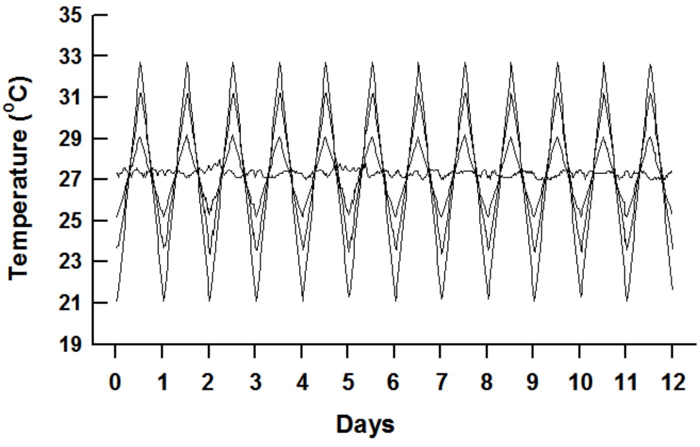

Fig. 1–Representative temperature profiles for a 12-day period for the four incubation temperature (27 °C, 27 ± 2 °C, 27 ± 4 °C and 27 ± 6 °C) regimes used in this study.

was assessed by chasing the hatchlings along a 2-m racetrack with one side transparent, which allowed videotaping with a NV-DS77 video camera (Panasonic, Japan). The racetrack was kept in a room at 30 ± 1 °C. Each hatchling was run twice with a 30 min rest between the two successive trials and, during the resting interval, it was placed back in the incubator. The tapes were later examined with a MGI VideoWave III software for PC (MGI Software Co., Canada) for sprint speed in the fastest 25 cm interval and the maximal sprinting distance traveled without stopping.

Following locomotor trials, we cooled the hatchlings to about 5 °C by placing them on a woody cooling box, and then measured them with Mitutoyo digital calipers. The box contained a substrate of ice (~50 mm depth), with a metal bracket where the temperature could be controlled by adjusting its distance to the ice substrate. 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 the tympanum), head width (taken at the posterior end of the mandible), fore-limb length (humerus plus ulna) and hind-limb length (femur plus tibia). We determined hatchling sex by pressing on both sides of the tail base using forceps to check for the presence or absence of hemipenes. Our diagnoses of gender in 2-month-old individuals were consistent with the results of sex determination at hatching.

Following morphological measurements, hatchlings were housed in the laboratory to measure growth rates. We individually numbered hatchlings, often at 10-day intervals, using a non-toxic waterproof ink, and then randomly moved them into one of five 50 cm × 40 cm × 40 cm plastic cages placed in a room at 20 ± 1 °C. A 60-W spotlight was mounted in each cage to allow thermoregulation for 14 h daily. Small mealworms, grasshoppers and house crickets were provided in excess and spread throughout the cage, such that hatchlings had free access to the food. We evaluated early growth (the first 60-day period) by weighing hatchlings at 30-day intervals.

1.4 Statistical analyses

All statistical procedures were performed in STATISTICA, version 6.0 for PC. Data on hatchlings of the same sex were pooled by clutch to avoid pseudo-replication. We used the *G* test to examine whether hatchability and the sex ratio of hatchlings differed among the four temperature treatments. We used two-way analysis of variance (ANOVA) and two-way analysis of covariance (ANCOVA) with egg mass as the covariate to examine whether egg mass, hatchling SVL and incubation length differed between the sexes and among the four temperature treatments. We used multivariate analysis of covariance (MANCOVA) with hatchling SVL as the covariate to examine the effects of sex, temperature treatment and their interaction on other hatchling morphological variables examined. Because the maximal distance and sprint speed both were independent of egg mass within each sex × temperature combination (all $P > 0.193$), we used multivariate analysis of variance (MANOVA) to examine the effects of sex, temperature treatment and their interaction on these two variables. We used repeated measures ANOVA with sex and temperature treatment as the between-subject factors and age (days since hatching) as the within-subject factor to analyze data on early growth. Prior to using parametric analyses, data were tested for homogeneity of variances using Bartlett’s test (univariate level) and/or Box’s M test (multivariate level), and for normality using the Kolmogorov-Smirnov test. Values are presented as mean ± standard error (SE), and statistical significance is assumed at $P < 0.05$.

2 Results

Mean values for egg mass did not differ among the four temperature treatments (two-way

ANOVA; $F_{3, 65} = 0.23$, $P = 0.876$), nor between the sexes ($F_{1, 65} = 0.22$, $P = 0.639$); the sex × temperature interaction was not a significant source of variation in egg mass ($F_{3, 65} = 1.12$, $P = 0.348$). Mean values for incubation length differed among the four treatments (two-way ANOVA; $F_{3, 65} = 12.06$, $P < 0.0001$), but not between the sexes ($F_{1, 65} = 0.48$, $P = 0.492$); the sex × temperature interaction did not affect incubation length ($F_{3, 65} = 0.10$, $P = 0.961$). The mean incubation length was longer in the 27 ± 6 °C treatment than in the other three treatments that did not differ from each other in the trait (Table 1). Hatching success ($G = 0.35$, $df = 3$, $P > 0.90$) and the sex ratio of hatchlings ($G = 2.30$, $df = 3$, $P > 0.50$) did not differ among the four treatments (Table 1).

Table 1 Effects of incubation thermal environments on incubation length, hatching success, and the sex ratio of hatchlings

Thermal treatments	Incubated eggs	Incubation length[1](d)	Hatching success (%)	Sex ratio (♀♀/♂♂)
27 °C	27	$38.4^{b} \pm 0.2$ (36.5–40.0)	74.0 (19/ 27)	11 / 8
27 ± 2 °C	30	$37.7^{b} \pm 0.3$ (35.2–39.0)	66.7 (20 / 30)	10 / 10
27 ± 4 °C	33	$38.1^{b} \pm 0.3$ (35.0–41.3)	84.8 (27 / 33)	18 / 9
27 ± 6 °C	32	$39.9^{a} \pm 0.2$ (38.5–41.2)	78.1 (25 / 32)	16 / 9

Data on incubation length are expressed as mean ± SE (range). Means with different superscripts differ significantly (Tukey's *post hoc* test, $\alpha = 0.05$).

[1] Data from the same clutches are blocked.

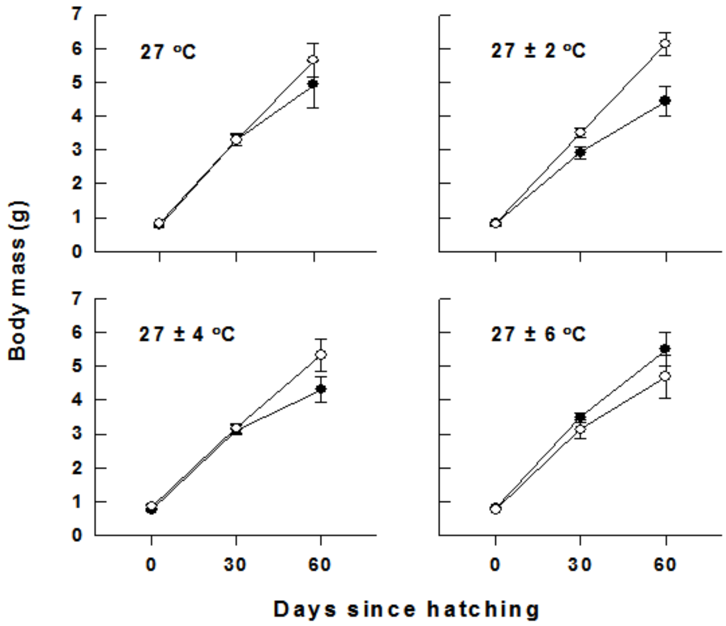

Fig. 2–Mean values (± SE) for hatchling growth in the first 60-day period. Solid dots represent females, and open dots represent males.

Table 2 Size, morphology and locomotor performance of hatchlings from eggs incubated under different thermal regimes

	27 °C		27 ± 2 °C		27 ± 4 °C		27 ± 6 °C	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
N	9	8	8	8	13	9	11	7
Egg mass	0.68 ± 0.03 (0.53–0.79)	0.76 ± 0.05 (0.55–0.98)	0.75 ± 0.04 (0.59–0.93)	0.71 ± 0.03 (0.58–0.83)	0.72 ± 0.03 (0.58–0.91)	0.77 ± 0.06 (0.58–1.16)	0.74 ± 0.03 (0.58–0.86)	0.70 ± 0.03 (0.59–0.84)
Body mass	0.77 ± 0.04 (0.58–0.94)	0.82 ± 0.05 (0.66–1.01)	0.83 ± 0.05 (0.66–1.00)	0.80 ± 0.03 (0.64–0.94)	0.77 ± 0.03 (0.62–0.96)	0.85 ± 0.05 (0.64–1.12)	0.83 ± 0.03 (0.58–0.94)	0.76 ± 0.02 (0.67–0.84)
Snout-vent length	34.0 ± 0.3 (32.5–35.7)	34.3 ± 0.7 (31.9–36.8)	34.4 ± 0.5 (32.7–36.7)	33.3 ± 0.5 (31.0–35.9)	34.0 ± 0.3 (32.6–37.2)	34.1 ± 0.6 (31.8–37.4)	34.5 ± 0.3 (33.3–36.0)	33.3 ± 0.5 (32.2–36.1)
Abdomen length	17.4 ± 0.2 (16.8–18.5)	17.4 ± 0.4 (16.2–19.5)	18.2 ± 0.5 (16.2–20.3)	16.6 ± 0.3 (15.4–18.6)	17.5 ± 0.3 (16.4–20.0)	16.7 ± 0.4 (14.3–18.3)	17.9 ± 0.3 (16.7–20.0)	17.1 ± 0.4 (15.5–19.1)
Tail length	46.6 ± 1.2 (40.0–53.0)	47.9 ± 2.0 (38.0–55.0)	46.9 ± 1.3 (43.0–52.0)	48.1 ± 1.0 (42.5–51.5)	46.9 ± 0.7 (43.0–50.5)	48.0 ± 1.6 (40.0–55.0)	46.2 ± 0.9 (41.0–52.0)	47.1 ± 1.8 (40.0–54.0)
Head length	7.5 ± 0.07 (7.2–7.9)	7.8 ± 0.1 (7.3–8.2)	7.6 ± 0.1 (7.2–8.2)	7.6 ± 0.09 (7.2–8.1)	7.6 ± 0.05 (7.4–8.1)	7.8 ± 0.1 (7.5–8.4)	7.6 ± 0.03 (7.4–7.7)	7.6 ± 0.08 (7.3–7.8)
Head width	5.3 ± 0.07 (5.1–5.6)	5.4 ± 0.1 (5.0–5.9)	5.3±0.08 (5.2–5.9)	5.4 ± 0.08 (5.2–5.8)	5.4±0.07 (5.1–5.9)	5.6 ± 0.1 (5.0–6.0)	5.3 ± 0.06 (5.1–5.7)	5.4 ± 0.07 (5.2–5.8)
Fore-limb length	7.4 ± 0.1 (6.9–7.7)	7.5 ± 0.1 (6.9–7.8)	7.6 ± 0.08 (7.3–8.0)	7.4 ± 0.1 (7.1–7.9)	7.5 ± 0.06 (6.9–7.7)	7.5 ± 0.1 (7.0–8.4)	7.5 ± 0.08 (7.0–8.1)	7.5 ± 0.04 (7.4–7.6)
Hind-limb length	9.2 ± 0.1 (8.7–9.7)	9.6 ± 0.2 (8.5–10.2)	9.5 ± 0.1 (8.7–10.0)	9.3 ± 0.1 (8.6–9.7)	9.6 ± 0.09 (8.8–10.0)	9.5 ± 0.2 (8.6–10.2)	9.4 ± 0.06 (9.1–9.7)	9.6 ± 0.1 (8.9–10.1)
The maximal distance (m)	0.87 ± 0.14 (0.40–1.80)	1.00 ± 0.14 (0.60–1.70)	0.85 ± 0.04 (0.70–1.05)	1.08 ± 0.13 (0.60–1.80)	0.93 ± 0.08 (0.60–1.60)	0.85 ± 0.11 (0.40–1.50)	0.80 ± 0.07 (0.50–1.20)	1.09 ± 0.16 (0.60–1.80)
Sprint speed	0.65 ± 0.03 (0.50–0.80)	0.74 ± 0.06 (0.45–0.98)	0.64 ± 0.05 (0.48–0.86)	0.76 ± 0.10 (0.51–1.28)	0.72 ± 0.06 (0.44–1.19)	0.76 ± 0.06 (0.52–1.05)	0.66 ± 0.04 (0.45–0.80)	0.65 ± 0.04 (0.55–0.83)

Data were expressed as mean ± SE (range). Morphological traits overall differed between the sexes, but not among the four treatments; Locomotor traits did not differ between the sexes, nor among the four treatments. The sex × temperature interaction was not a significant source of variation in all the examined traits (see text for details)

Table 2 shows size, morphology and locomotor performance of hatchlings from eggs incubated under different thermal regimes. Mean values for SVL differed between the sexes, with females being the larger sex (two-way ANCOVA with egg mass as the covariate; $F_{1, 64} = 8.05$, $P < 0.005$); Mean values for SVL did not differ among the four treatments ($F_{3, 64} = 0.52$, $P = 0.667$), and the sex × temperature interaction was not a significant source of variation in SVL ($F_{3, 65} = 0.10$, $P = 0.961$). Other hatchling morphological traits (body mass, abdomen length, tail length, head length, head width, fore-limb length and hind-limb length) overall differed between the sexes (Wilks' $\lambda = 0.621$, $df = 7, 58$, $P < 0.0002$), but not among the four treatments (Wilks' $\lambda = 0.716$, $df = 21, 167$, $P = 0.488$); the sex × temperature interaction was not a significant source of variation in the examined traits (Wilks' $\lambda = 0.618$, $df = 21, 167$, $P = 0.102$). Specifically, female hatchlings were shorter in head length and width but longer in SVL as well as abdomen length than males with the same SVL (all $P < 0.025$), whereas the other morphological traits did not differ between the sexes (all $P > 0.242$). The two locomotor traits (the maximal distance and sprint speed; Table 2) did not differ between the sexes (Wilks' $\lambda = 0.943$, $df = 2, 64$, $P = 0.155$), nor among the four treatments (Wilks' $\lambda = 0.933$, $df = 6, 128$, $P = 0.610$); the sex × temperature interaction did not affect these two locomotor traits (Wilks' $\lambda = 0.919$, $df = 6, 128$, $P = 0.483$).

Four female and three male hatchlings died in the first 60-day period, and dead hatchlings could be found in each of the four treatments. Post-hatching growth was very evident ($F_{2, 116} = 471.87$, $P < 0.0001$) (Fig. 2), but did not differ between the sexes ($F_{1, 58} = 3.06$, $P = 0.086$) nor among the four treatments ($F_{2, 58} = 0.47$, $P = 0.704$). Of the interactions involved, only that between sex and age had a marginally significant effect on growth rate ($F_{2, 116} = 3.16$, $P = 0.046$).

Mass gain in the first 60-day period was unrelated to body size at hatching within each sex × temperature treatment combination (linear regression analysis; all $P > 0.191$).

3 Discussion

There are four main findings in this study: (1) hatchlings incubated under the four temperature regimes did not differ from each other in any examined trait except birth date; (2) interactions that included temperature treatment (the magnitude of temperature variation) did not affect any trait examined; (3) the mean incubation length was longer in the 27 ± 6 °C treatment than in the other three treatments; and (4) female hatchlings were shorter in head length and width but longer in SVL as well as abdomen length than males derived from the same sized egg. The first two findings allow us to conclude that incubation temperature fluctuation affects incubation length but not other examined hatchling phenotypes in *L. agilis*. This conclusion is overall consistent with that drawn in other reptiles such as *Pelodiscus sinensis* (Chinese soft-shelled turtle; Du and Ji 2003; Ji *et al.* 2003), *Eremias argus* (Mongolian racerunner; Hao *et al.* 2006), *E. chinensis* (Chen *et al.* 2003), *Heteronotia binoei* (Binoe's prickly gecko; Andrewartha *et al.* 2010), *Rhabdophis tigrinus lateralis* (red-necked keelback snake; Chen and Ji 2002), *Ptyas mucosus* (common ratsnake; Lin and Ji 2004), *N. atra* (Lin *et al.* 2008) and *Xenochrophis piscator* (checkered keelback snake; Lu *et al.* 2009). In all these species, incubation temperature fluctuation has no role in modifying hatchling traits as long as eggs are not exposed to extreme temperatures for prolonged periods of time.

Eggs of *L. agilis* have been incubated at five constant temperatures between 22 °C and 31 °C (Jensen 1981; Olsson *et al.* 1996; Olsson and Shine 1997). The temperatures used in earlier studies are thought to be higher than in nature, as seen by the fact that the mean incubation length (~60 days) at 22 °C is much shorter than an incubation time (~100 days) found in nature, which corresponds to a mean temperature of about 16 °C under the assumption that Q_{10} is constant across the range of temperatures suitable for embryonic development (Jensen 1981). Given that hatching successes are high at these temperatures except 31 °C (Jensen 1981), it seems likely that, as in other lacertid lizards so far studied (Van Damme *et al.* 1992; Lin and Ji 1998; Pan and Ji 2001; Xu *et al.* 2005; Du and Ji 2006; Hao *et al.* 2006; Wu and Xu 2007; Rodríguez-Díaz *et al.* 2010), exposure of eggs to temperatures higher than 30 °C for prolonged periods of time has an adverse effect on embryonic development in *L. agilis*.

High incubation temperatures affect embryonic development differently than low temperature in reptiles. For example, incubation at extremely high temperatures not only increases embryonic mortality but also affects several aspects of a hatchling’s morphology and performance, whereas low incubation temperatures, though slowing or even arresting embryonic development, often have no adverse effects on hatching success and hatchling traits (Lin *et al.* 2010). Moreover, it is widespread among oviparous reptiles that incubation at high temperatures close to the upper thermal threshold for embryonic development results in a less amount of yolk converted to somatic tissue such that hatchlings incubated at these temperatures have smaller carcasses (and thus, smaller body dimensions) but larger residual yolks (Lin *et al.* 2010). In the present study, only eggs in the 27 ± 6 °C treatment experienced temperatures higher than 30 °C, which accounted for about 23.4% of total temperature recordings (Fig. 1). However, contrary to what was expected, the 27 ± 6 °C treatment did not reduce hatching success, nor did it produce hatchlings that differed from those in other three treatments in any trait examined. This finding therefore adds evidence that daily exposure of eggs to extreme temperatures for brief periods of time may not necessarily increase embryonic mortality and result in major phenotypic modifications in reptiles (Lu *et al.* 2009).

There are several studies showing the importance of incubation temperature fluctuation in inducing phenotypic variation in reptiles. For example, fluctuating temperatures affect locomotor performance in *Apalone mutica* (smooth soft-shelled turtle; Ashmore and Janzen 2003), *Takydromus septentrionalis* (northern grass lizard; Du and Ji 2006), *Bungarus multicintus* (multi-banded krait; Ji *et al.* 2007a) and *Elaphe obsolete* (black ratsnake; Patterson and Blouin-Demers 2008). In two TSD turtles such as *Trachemys scripta* (red-eared slider turtle; Les *et al.* 2007) and *Chrysemys picta* (painted turtle; Paitz *et al.* 2010), incubation temperature fluctuation affects offspring sex ratios. Nonetheless, in none of these studies could incubation temperature fluctuation be translated into marked differences in hatchling phenotypes (except the sexual phenotype in TSD species) with much certainty because thermal fluctuations may result in exposure of eggs to temperatures that have a key role in modifying hatchling traits. In *X. piscator*, for example, hatchlings incubated at 30 °C and temperatures fluctuating within the range of 20-36 °C (mean = 27.7 °C) have smaller body sizes as compared with those incubated at 24 °C and 27 °C (Lu *et al.* 2009). This temperature-induced modification of body size is highly consistent with the finding from an earlier study of *X. piscator* where eggs incubated at high constant temperatures (30 °C or higher) produce hatchlings with smaller carcasses and therefore shorter total body lengths (SVL and tail length) as compared with those incubated at moderate temperatures (Ji *et al.* 2001). That thermal fluctuations have no direct role in determining the phenotype of hatchlings has also been found in viviparous reptiles such as *Mabuya multifasciata* (many-lined sun skink; Ji *et al.* 2007b) and *Gloydius brevicaudus* (short-tailed pit viper; Gao *et al.* 2010). Gravid females of these two species regulate body temperature more precisely and therefore maintain less variable 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 of temperatures optimal for embryonic development is narrow (Ji *et al.* 2007b; Gao *et al.* 2010). With currently available data, we believe that the mean rather than the variability of incubation temperatures has a direct role in affecting the phenotype of hatchlings in reptiles.

As in other reptile taxa (Lin *et al.* 2010 and references therein), the response of developmental rate to constant temperature results in a curvilinear relationship in lacertid lizards,

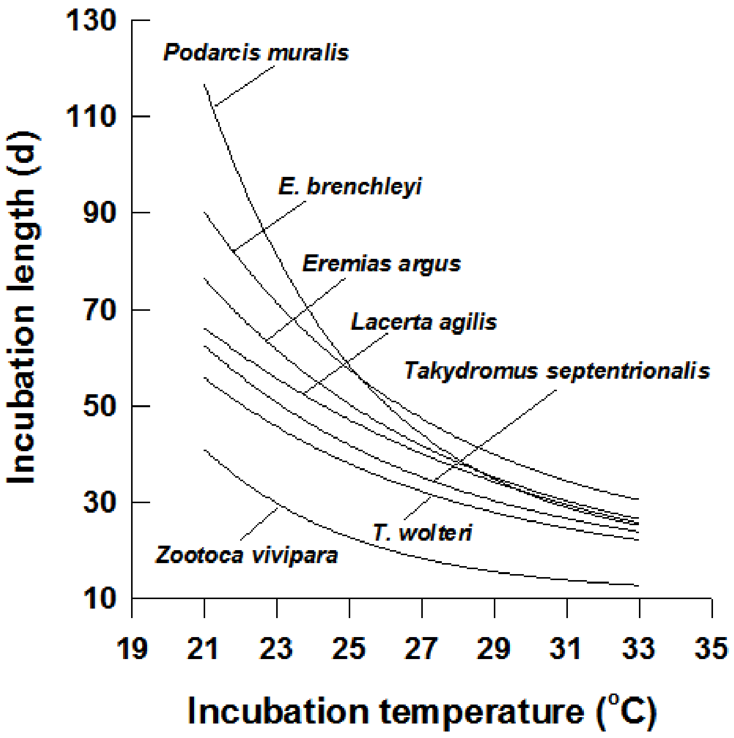

Fig. 3–The relationship between incubation length (mean) and constant incubation temperature in lacertid lizards, including *Eremias argus* (Hao *et al.* 2006), *E. brenchleyi* (Xu *et al.* 2005), *Lacerta agilis* (Jensen 1981), *Podarcis muralis* (Van Damme *et al.* 1992; Ji and Braña 1999; Braña and Ji 2007), *Takydromus septentrionalis* (Lin and Ji 1998; Du and Ji 2006; Du and Feng 2008), *T. wolteri* (Pan and Ji 2001; Wu and Xu 2007) and *Zootoca vivipara* (Rodríguez-Díaz *et al.* 2010).

including *L. agilis* (Fig. 3). When temperatures fluctuate sinusoidally in a range suitable for embryonic development, an acceleration in developmental rate with increased temperatures can be equally offset by a deceleration in developmental rate with decreasing temperatures (Les *et al.* 2009). However, as developmental rate increases at an ever-decreasing rate as temperature increases across the range of viable incubation temperatures (Fig. 3), fluctuating temperatures should result in an increase in incubation length when compared to constant-temperature incubations conducted at the mean of the fluctuating regime. For eggs of *L. agilis* incubated at constant temperatures, the mean incubation length decreases by about 26 days at the changeover from 21 °C to 27 °C and by about 14 days at the changeover from 27 °C to 33 °C (Fig. 1). The difference (~12 days) between these two changeovers is greater than that between any two equiinterval changeovers in a temperature range narrower than 21-33 °C (e.g., ~5 days in the range of 23-21 °C, and ~1 day in the range of 25-29 °C). In the present study, however, incubation of eggs at 27 ± 6 °C only increased incubation length by about 1.5 days as compared with the 27 °C treatment (Table 1), primarily because temperatures fluctuated in a sinusoidal rather than a saltatorial way. This observation, together with the finding that eggs incubated under the other two fluctuating regimes did not differ from those incubated at 27 °C in incubation length (Table 1), suggest that both the way and the magnitude of temperature variation can affect incubation length.

Female hatchlings were shorter in head length and width but longer in SVL as well as abdomen length than male hatchlings derived from the same sized eggs. Thus, as in other lacertid lizards including *E. argus* (Hao *et al.* 2006), *Podarcis muralis* (common wall lizard; Braña and Ji

2000), *Takydromus wolteri* (white-striped grass lizard; Pan and Ji 2001), *T. septentrionalis* (Du and Ji 2006) and *Zootoca vivipara* (common lizard; Rodríguez-Díaz *et al.* 2010), sexual dimorphism occurs at hatching in *L. agilis*. Interestingly, in none of these species are female hatchlings smaller in SVL and abdomen length and larger in head size, limb length and tail length than males derived from the same sized eggs. It seems likely that the pattern of sexual dimorphism at hatching is similar among lacertid lizards.

In summary, our data show that incubation temperatures fluctuating sinusoidally within the range of 21-33 °C affect incubation length but not hatching success in *L. agilis*, and confirm that intermittent exposure of reptile eggs to extreme temperatures may not necessarily increase embryonic mortality. Our data show that both the type and the magnitude of temperature variation can affect incubation length in reptiles. We found no evidence for phenotypic divergence in responses to temperature fluctuations during incubation, and therefore suggest that temperature variation *per se* has no role in influencing the phenotype of hatchlings in *L. agilis*.

Acknowledgements

The work was carried out in compliance with the current laws of China, and was supported by grant from National Science Foundation of China (Project No. 30670281) and Chinese Ministry of Education (Project No. 20103207120009). The authors would like to thank Jian-Fang Gao, Hong-Liang Lu, Yan-Fu Qu and Qun-Li Zhang for their help during the research.

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波动孵化温度影响捷蜥孵化期但不影响其形态，运动及幼体生长

李宏，周宗师，丁国骅，计翔

（南京师范大学生命科学学院，南京 210046）

摘要：许多研究孵化温度和波动的孵化温度对爬行动物新生幼体的表型潜在修饰作用的结果表明这种效应存在着物种间的差异，但并没有从本质上区分温度波动和极限温度之间的差异。为检测波动孵化温度是否对后代表型特征变化起关键作用，将捷蜥的卵置于 27 度，以及以 27 度为平均温度分别上下波动 2，4，6 度四种热处理下孵化。实验结果表明：（1）四种热处理下孵出的幼体在所有形态和生理特征上均没有显著差异；（2）包括温度处理本身的交互作用对任何检测到的特征均没有修饰作用；（3）波动振幅为 6 度处理下的平均孵化期长度要长于其他三个热处理；（4）雌性幼体的头部大小要比同等大小卵孵化出的雄性幼体小，但体长和腹长要比雄性幼体长。当前研究数据表明波动温度的类型和尺度只影响孵化期长度，没有证据显示温度变异的方式和尺度能影响孵化期长度。没有证据显示对孵化期间的波动温度响应有表型差异，表明孵化温度变化对捷蜥孵出幼体没有作用。

关键词：蜥蜴科；捷蜥；热波动；孵化温度；后代表型；孵化期；生长

中图分类号：Q958.1