Phenotypic variation in hatchling Mongolian racerunners *Eremias argus* from eggs incubated at constant versus fluctuating temperatures*

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Abstract　We used the Mongolian racerunner *Eremias argus* as a model animal to evaluate the effects of constant versus fluctuating incubation temperatures on hatching success and hatchling phenotypes. Eggs were incubated under four constant［24，27，30 and 33（±0.3)℃］and one fluctuating temperature regimes. Hatching success did not differ among treatments，and incubation temperature did not affect the sexual phenotype of hatchlings. Incubation length decreased exponentially as incubation temperature increased，and eggs incubated at fluctuating temperatures took a longer time to complete development than did those incubated at constant temperatures with the same mean. Of the hatchling phenotypes examined，body dry mass，carcass dry mass，residual yolk dry mass and locomotor performance of hatchlings were more likely to be affected by incubation temperature. Overall，locomotor performance was best in the low temperature treatments（24℃ and 27℃）and worst in the highest temperature treatment（33℃），with the moderate temperature treatments（30℃ and fluctuating temperatures）in between. Our data show that：（1）daily exposure of eggs to extreme temperatures that are potentially lethal to embryos for brief periods does not have detectable adverse effects on hatching success and morphological phenotypes in *E. argus*；and（2）thermal fluctuations exert no positive effects on locomotor performance of hatchlings but influence incubation length differently than constant temperatures with the same mean［*Acta Zoologica Sinica* 52（6）：1049－1057，2006］.

Key words　Reptilia，Lacertidae，*Eremias argus*，Egg incubation，Hatching success，Hatchling phenotype，Locomotor performance

恒定和波动温度下丽斑麻蜥孵出幼体的表型变异*

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摘　要　作者以丽斑麻蜥（*Eremias argus*）为模型动物研究恒定和波动孵化温度对孵化成功率和孵出幼体表型的影响。卵在四个恒定［24，27，30 and 33（±0.3)℃］、一个波动温度下孵化。不同温度处理下的孵化成功率相同，但孵出幼体表型不同。孵化期随孵化温度升高呈指数式缩短；在相同平均温度下，波动温度孵化卵的孵化期比恒温孵化卵长。在所有被检表型特征中，幼体的干重、剩余卵黄干重和运动表现更易受孵化温度影响。总

体而言，低温（24℃、27℃）孵出幼体运动表现最佳，高温（33℃）孵出幼体最差、温和温度（30℃和波动温度）孵出幼体居中。本文研究数据显示：（1）丽斑麻蜥卵每日短期暴露于潜在致死的极端温度下对孵化成功率和孵出幼体形态特征无明显的不利效应；（2）温度波动对孵出幼体运动表现无促进作用，对孵化期的影响则不同于平均值相同的恒定温度［动物学报 52（6）：1049－1057，2006］。

关键词　爬行纲　蜥蜴科　丽斑麻蜥　卵孵化　孵化成功率　幼体表型　运动表现

In reptiles，as in other vertebrate and invertebrate taxa（Meats，1984；Ratte，1985；Deeming and Ferguson，1991；West-Eberhard，2003），temperature regimes experienced by embryos affect not only embryonic survivorship and rates of development，but also morphological，physiological and behavioral phenotypes of the hatchling（Joanen et al.，1987；Burger，1991，1998；Allsteadt and Lang，1995；Packard and Phillips，1995；Booth，1999，2000；Rhen and Lang，1999a，b；Braña and Ji，2000）. In species with temperature-dependent sex determination，incubation or gestation temperature also affects the sexual phenotype（Janzen and Paukstis，1991；Lang and Andrews，1994；Viets et al.，1994；Robert and Thompson，2001；Wapstra et al.，2004）. Most studies of reptiles have been based on embryos developing at constant temperatures and，thus，whether and how fluctuating temperatures influence developing embryos differently than constant temperatures remain largely unknown.

Temperatures within natural nests are rarely constant but fluctuate daily and seasonally，with the mean and the amplitude of thermal fluctuations depending on locality，microhabitat，year and season. Therefore，the thermal impacts demonstrated in constant-temperature incubation often do not reflect what truly occurs in nature（Overall，1994；Shine and Harlow，1996；Shine et al.，1997a，b；Valenzuela，2001）. Recent work on incubation of reptilian eggs has preferred the simulation of nest thermal environments rather than applying constant temperature regimes. Data generated from this approach show that eggs of different species respond differentially to the mean and/or the variance of incubation temperatures（Overall，1994；Georges et al.，1994；Shine and Harlow，1996；Andrews et al.，2000；Webb et al.，2001；Chen and Ji，2002；Chen et al.，2003；Ji et al.，2003；Asmore and Janzen，2003；Du and Ji，2006）. However，fluctuating temperatures influence incubation length differently than constant temperatures with the same mean in some species of reptiles（Overall，1994；Shine and Harlow，1996；Asmore and Janzen，2003）but not in others（Georges et al.，1994；Andrews et al.，2000；Webb et al.，2001）.

The Mongolian racerunner Eremias argus studied herein is a small sized［to 70 mm snout-vent length（SVL）］oviparous lacertid lizard that ranges from northern China（southward to Jiangsu and westward to Qinghai）to Russia（region of Lake Baikal），Mongolia and Korea（Zhao，1999）. Females lay eggs in shallow nests where temperatures vary pronouncedly in response to short-term environmental variation of thermal flux and，thus，offer an ideal model system in which to investigate phenotypically plastic responses of embryos to thermal fluctuations. In this study，we incubated eggs under four constant and one fluctuating temperature regimes to address three questions：（1）does daily exposure of eggs to extreme temperatures that are harmful or even lethal to embryos for brief periods have detrimental effects on hatching success and hatchling phenotypes？（2）what hatchling phenotypes are more likely to be affected by incubation temperature？（3）do fluctuating temperatures influence developing embryos differently than constant temperatures？

1 Materials and methods

1.1 Collection and animal care

Adult lizards（＞47 mm SVL）were collected by hand or noose in late April 2004 from several localities in the vicinity of Linfen（36°06′N，111°33′E），Shanxi，northern China，and were transported to our laboratory in Hangzhou，where 16－20 individuals（females/males was c. 2/1）were housed together in each of the four 90 cm×65 cm×50 cm（length×width×height）communal cages with 5 cm depth sand and pieces of clay tile. These cages were placed in a room where air temperatures were never outside the range of 20－28℃. A 100-W light bulb，suspended at one end of each cage，created a thermal gradient ranging from ambient room temperature to 55℃ for 12 h to allow thermoregulation during the photophase. Lizards were fed mealworms Tenebrio molitor and water enriched with vitamins and minerals ad libitum. Females with shelled oviductal eggs were removed from the communal cages，and housed individually in 20 cm×15 cm×20 cm egg-laying cages with 4 cm depth moist sand and a 20-W spotlight mounted in each cage to allow thermoregulation.

1.2 Egg collection and incubation

Eggs were collected，measured and weighed no later than three hours after being laid，thereby avoiding any uncertainty about the initial mass due to loss or gain of water（Lin and Ji，1998）. The viability of freshly laid eggs was judged by the presence of a small embryonic disc using a spotlight. Post-oviposition fe-

males were measured，weighed and marked by painting before they were returned to the communal cages where they remained until they again carried shelled oviductal eggs，at which time they were once again transferred to the egg-laying cages. Ten freshly laid eggs sampled randomly from different clutches were dissected for identification of embryonic stage at oviposition，according to the criteria proposed by Dufaure and Hubert（1961）.

Eggs were incubated under five temperature regimes（thermal treatments），and eggs from the same clutch were never incubated under the same thermal condition. One plastic container（30 cm × 15 cm × 10 cm）was used to hold all the eggs，which were separated from each other by PVC tubes（inner diameter = 2 cm）for accurate identification of the e-mergent young，in a given thermal treatment. The containers holding eggs contained known amounts of vermiculite and distilled water（1 g water：1 g dried vermiculite）to produce approximately − 220 kPa water potential（Ji and Braña，1999），and were covered with a perforated plastic membrane to retard water loss. Eggs were half-buried in the substrate，with the surface near the embryo being exposed to air inside the container. We weighed containers every other day and，if necessary，added distilled water to compensate for small loss of water due to evaporation and absorption of water by the incubating eggs.

Four containers were individually assigned to four different Shellab incubators（Sheldon MFG Inc，USA）inside which temperatures were controlled at 24，27，30 and 33（ ± 0.3）℃，respectively. We moved containers among shelves daily according to a predetermined schedule to minimize any effects of thermal gradients inside the incubator. The remaining container（hereafter F-treatment）was placed in a 60 cm × 60 cm × 30 cm chamber buried 15 cm below the ground surface in the bush-covered backyard of our laboratory，thereby mimicking thermal conditions in natural nests. A tinytalk datalogger（Gemini Pty，Australia）programmed to record temperature at one-hour intervals was placed in the chamber throughout the experiment. Mean，minimal and maximal temperatures experienced by individual eggs in the F-treatment varied from 26.5 − 31.6℃，19.5 − 21.1℃ and 36.1 − 37.7℃，respectively.

1.3　Incubation length and hatchling phenotypes

Wet body mass was taken for each newly e-merged young，and the incubation length was recorded as the number of days to pipping. 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℃，which was controlled by placing hatchlings in an incubator at the correspon-

dent 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，the maximal distance traveled without stopping（hereafter the maximal distance）and the number of stops in the racetrack.

After examination of locomotor performance，hatchlings were killed by freezing to − 15℃ for later collection of morphological data. Morphological measurements taken for each killed 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），hind-limb length（femur plus tibia）and the number of ventral scales. 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 un-equivocal sex assignation of males（ Ji and Braña，1999 ）. After taking these measurements，each hatchling was separated into carcass，fat bodies and residual yolk. These components were dried in an oven at 60℃ to constant mass，and then weighed.

1.4　Data analyses

All data were tested for normality（Kolmogorov-Smirnov test）and homogeneity of variances（Bartlett test），and $\log_e$ transformations were performed when necessary to satisfy the assumptions for parametric tests. 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 ± 1 standard error，and the significance level is set at $\alpha = 0.05$.

2　Results

Females laid up to two clutches per breeding season stretching from late April to early August. Clutch size averaged 3.0（$SE = 0.1$；range $= 2 − 6$，$n = 45$）eggs in the first clutch and 3.1（$SE = 0.1$；range $= 2 − 5$，$n = 38$）eggs in the second clutch，and egg mass averaged 0.43（$SE = 0.01$；$range = 0.31 − 0.57$，$n = 45$）g in the first clutch and 0.42（$SE = 0.01$；$range = 0.31 − 0.58$，$n = 38$）g in the second clutch. Only eggs of the first clutch were used in this investigation. Of the ten embryos identified at oviposition，five were at Stage 26，three at Stage 25 and one at Stage 23 in Dufaure and Hubert's（1961）developmental series.

2.1　Hatching success，incubation length and the sex ratio of hatchlings

Hatching success did not differ significantly among treatments（$G = 1.32$，$df = 4$，$P > 0.75$；Table 1）. Incubation length，which was not correlated with initial egg mass within each treatment（all $P > 0.10$），differed considerably among treatments（Kruskal-Wallis test，$H_{4, n=98} = 88.63$，$P < 0.0001$）. Incubation length decreased exponentially as incubation temperature increased in eggs incubated at constant temperatures，with the mean incubation length being shortened by 14.1 days from 24℃ to 27℃，11.1 days from 27℃ to 30℃，and 2.6 days from 30℃ to 33℃（Table 1）. Incubation length was

negatively correlated with the mean temperature during incubation in the F-treatment（$F_{1, 14} = 127.35$，$P < 0.0001$；Fig.1）. Eggs incubated at fluctuating temperatures differed significantly from eggs incubated at constant temperatures with the same mean in incubation length（ANCOVA，$F_{1, 17} = 20.49$，$P < 0.0003$），with the mean incubation length being longer in the F-treatment at any given temperature. More female hatchlings（59 females / 39 males）were produced in this study，but the sex ratio of hatchlings did not differ significantly among treatments（$G = 1.43$，$df = 4$，$P > 0.75$）（Table 1）.

Table 1　Incubation length，hatching success and the sex ratio of hatchling _E. argus_ derived from eggs incubated under different temperature regimes

Thermal treatment	Number of incubated egg	Incubation length（d）	Hatching success（%）	Sex ratio（F/M）
24℃	30	56.0±0.6（50.0−59.4）	76.7（23/30）	14/9
27℃	18	41.9±0.5（39.5−47.0）	83.3（15/18）	9/6
30℃	18	30.8±0.3（28.9−33.0）	88.9（16/18）	9/7
33℃	34	28.2±0.4（23.9−30.9）	82.4（28/34）	19/9
F	23	37.2±1.0（32.8−46.0）	69.6（16/23）	8/8

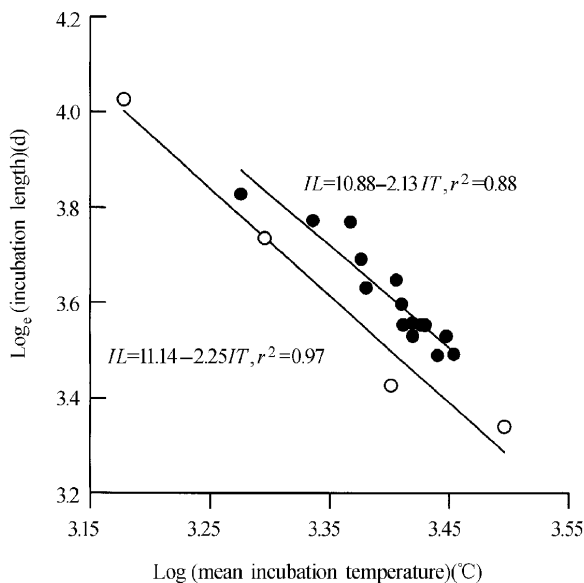Data on duration of incubation are expressed as Mean±SE（range）. F: female. M: male

Fig.1　Linear regressions of incubation length on the mean incubation temperature
Solid dots: eggs incubated at fluctuating temperatures; open dots: eggs incubated at constant temperatures. Regression lines are given in the figure

2.2　Hatchling phenotypes

Preliminary two-way ANOVAs（with sex and thermal treatment as the factors）on residuals from the regressions of the involved hatchling variables on initial egg mass did not reveal between-sex differences in body mass and hatchling components（all $P > 0.15$），so we pooled data for both sexes. Incubation

temperature significantly affected hatchling dry mass，carcass dry mass and residual yolk dry mass but not hatchling wet mass and fatbody dry mass，with dry body mass and carcass dry mass being apparently smaller in the 33℃ treatment than in other four treatments（Table 2）. More yolks remained unutilized at hatching at the two high incubation temperatures（30℃ and 33℃）（Table 2）.

Except for the number of ventral scales，morphological phenotypes did not differ among treatments（Table 3）. The number of ventral scales was greatest in the 33℃ treatment and smallest in the 27℃ and F treatments，with the 24℃ and 30℃ treatments in between（Table 3）. Female hatchlings were larger in SVL but smaller in head length，and had more ventral scales than male hatchlings from the same sized eggs（Table 3）.

None of the three locomotor variables was correlated with hatchling SVL and differed between sexes（all $P > 0.10$）. One-way ANOVA with thermal treatment as the factor revealed that sprint speed（$F_{4, 93} = 6.01$，$P < 0.0003$），the maximal distance（$F_{4, 93} = 12.65$，$P < 0.0001$）and the number of stops all differed significantly among treatments. Sprint speed was slower in the 33℃ and faster in other four treatments（Fig.2A）. The maximal length was longer in the 24℃ and 27℃ treatments and shorter in the remaining three treatments（Fig.2B）. The number of stops was greatest in the 33℃ treatment and smallest in 24℃ and 27℃ treatments，with the 30℃ and F treatments in between（Fig.2C）.

Table 2 Descriptive statistics for body mass of hatchling *E . argus* derived from eggs incubated under different temperature regimes（all mass units are in mg）

	Thermal treatments					*F* values and significance levels
	24℃	27℃	30℃	33℃	F	
N	14F/9M	9F/6M	9F/7M	19F/9M	8F/8M	
Initia legg mass	423.5±12.7 （317.0－568.0）	386.7±12.5 （295.0－463.0）	407.7±13.4 （321.0－498.0）	415.1±10.9 （329.0－586.0）	382.8±12.9 （260.0－475.0）	$F_{4, 93}=1.91$，$P=0.116$
Wet body mass	548.4±17.7 （360.8－714.1）	491.2±16.0 （345.3－582.5）	526.0±20.1 （338.7－640.5）	508.7±11.1 （387.4－680.0）	476.1±15.7 （342.6－579.0）	$F_{4, 92}=1.90$，$P=0.117$
Dry body mass	102.2±4.1 （64.4－138.9）	92.5±3.2 （65.2－109.4）	103.0±4.7 （65.9－140.7）	92.6±2.9 （62.4－131.4）	89.7±3.3 （61.4－108.2）	$F_{4, 92}=2.50$，$P=0.047$ 24[ab], 27[ab], 30[a], 33[b], F[ab]
Carcass dry mass	95.2±3.4 （63.8－126.6）	88.4±2.9 （64.5－104.9）	92.6±3.7 （62.5－119.1）	83.7±2.2 （55.9－115.0）	85.0±3.0 （56.5－102.0）	$F_{4, 92}=4.35$，$P<0.003$ 24[a], 27[a], 30[a], 33[b], F[ab]
Fatbody dry mass	2.3±0.4 （Nil－6.7）	2.0±0.3 （0.7－4.8）	2.6±0.4 （Nil－5.4）	1.6±0.2 （Nil－4.1）	1.7±0.3 （Nil－3.7）	$F_{4, 93}=1.89$，$P=0.118$
Residual yolk dry mass	4.6±0.9 （Nil－15.4）	2.1±0.6 （Nil－6.3）	7.7±1.4 （Nil－19.8）	7.4±1.1 （Nil－23.0）	3.0±0.7 （Nil－7.7）	$F_{4, 93}=4.86$，$P<0.002$ 24[ab], 27[b], 30[a], 33[a], F[ab]

Data are expressed as Mean±*SE*（range）. *F* values of ANOVAs（for initial egg mass，fatbody dry mass and residual yolk day mass）or ANCOVAs（for the remaining variables with initial egg mass as the covariate），and significance levels are indicated in the table. Means corresponding to different thermal treatments with different superscripts differ significantly（Tukey's post-hoc test，$\alpha=0.05$；a＞b）

Table 3 Morphological phenotypes of hatchling *E . argus* derived from eggs incubated under different temperature regimes（all length units are in mm）

	Sex	Thermal treatments					Effects		
		24℃	27℃	30℃	33℃	F	Sex	Temperature	Interaction
N		14F/9M	9F/6M	9F/7M	19F/9M	8F/8M			
Snout-vent length	F	27.0±0.3 25.2－30.0	26.7±0.5 23.6－28.5	27.1±0.4 25.9－29.3	26.5±0.4 23.3－29.6	27.1±0.4 24.9－29.4	$F_{1, 87}=5.59$, $P=0.020$; F＞M	$F_{4, 87}=1.55$, $P=0.196$	$F_{4, 87}=1.89$, $P=0.127$
	M	26.8±0.4 25.1－28.9	26.6±0.3 25.2－27.7	26.0±0.7 22.6－28.1	26.0±0.4 24.4－27.4	25.7±0.4 23.8－28.0			
Tail length	F	33.4±0.7 26.2－36.2	33.3±0.7 29.5－35.2	34.0±0.5 31.5－36.8	32.3±0.8 24.9－38.1	33.4±1.0 28.2－36.9	$F_{1, 87}=3.22$, $P=0.076$	$F_{4, 87}=1.87$, $P=0.123$	$F_{4, 87}=1.79$, $P=0.138$
	M	34.5±1.0 30.0－38.1	31.6±1.2 27.7－36.1	35.5±2.4 23.2－42.3	33.8±0.8 31.2－39.0	31.8±1.1 27.7－36.0			
Head length	F	7.0±0.1 6.5－7.4	6.6±0.1 6.1－7.1	6.7±0.1 6.3－7.1	6.9±0.1 6.1－7.5	7.0±0.1 6.7－7.2	$F_{1, 87}=5.88$, $P=0.017$, F＜M	$F_{4, 87}=0.74$, $P=0.570$	$F_{4, 87}=4.45$, $P<0.003$
	M	6.9±0.1 6.2－7.3	7.0±0.1 6.6－7.3	6.9±0.1 6.1－7.2	6.7±0.1 6.0－7.3	6.8±0.1 6.4－7.3			
Head width	F	5.1±0.1 4.3－5.9	5.3±0.1 4.8－5.5	5.0±0.1 4.7－5.2	4.9±0.05 4.6－5.2	5.2±0.1 5.1－5.6	$F_{1, 87}=2.62$, $P=0.109$	$F_{4, 87}=0.76$, $P=0.551$	$F_{4, 87}=3.72$, $P<0.008$
	M	5.2±0.1 4.9－5.8	5.1±0.1 4.8－5.4	5.2±0.1 4.9－5.5	5.2±0.1 4.7－5.5	5.0±0.1 4.5－5.5			
Fore-limb length	F	7.1±0.1 6.3－8.1	7.0±0.2 6.4－7.9	7.1±0.1 6.5－7.8	7.0±0.1 6.0－8.0	6.9±0.2 5.9－7.4	$F_{1, 87}=0.44$, $P=0.510$	$F_{4, 87}=0.77$, $P=0.551$	$F_{4, 87}=0.61$, $P=0.658$
	M	7.1±0.2 6.5－7.8	7.1±0.2 6.3－7.8	6.9±0.2 5.6－7.3	6.8±0.1 6.1－7.5	6.7±0.1 6.1－7.4			
Hind-limb length	F	9.7±0.2 8.6－10.8	9.9±0.2 8.7－10.7	10.2±0.2 9.4－11.0	9.9±0.2 8.7－11.1	9.7±0.2 8.7－10.8	$F_{1, 87}=0.01$, $P=0.931$	$F_{4, 87}=2.07$, $P=0.090$	$F_{4, 87}=0.33$, $P=0.854$
	M	9.7±0.2 8.1－10.4	9.8±0.3 8.9－10.7	9.7±0.3 7.8－10.6	9.7±0.2 8.9－11.0	9.4±0.2 8.2－10.6			
Ventral scale	F	31.6±0.7 28－37	30.2±0.4 28－33	31.9±0.8 28－36	33.3±0.7 26－40	30.3±0.5 28－33	$F_{1, 88}=6.75$, $P=0.011$; F＞M	$F_{4, 88}=3.91$, $P<0.006$; 24[ab], 27[b], 30[ab], 33[a], F[b]	$F_{4, 88}=1.14$, $P=0.344$
	M	29.9±0.7 28－34	29.3±0.7 27－32	30.4±0.7 27－33	30.2±0.6 27－34	29.2±0.4 27－31			

Data are expressed as Mean ±*SE*（range）. *F* values of two-way ANOVA（for ventral scale）or ANCOVAs（for the remaining variables with initial egg mass as the covariate），and significance levels are indicated in the table. Means corresponding to different thermal treatments with different superscripts differ significantly（Tukey's post-hoc test，$\alpha=0.05$；a＞b）
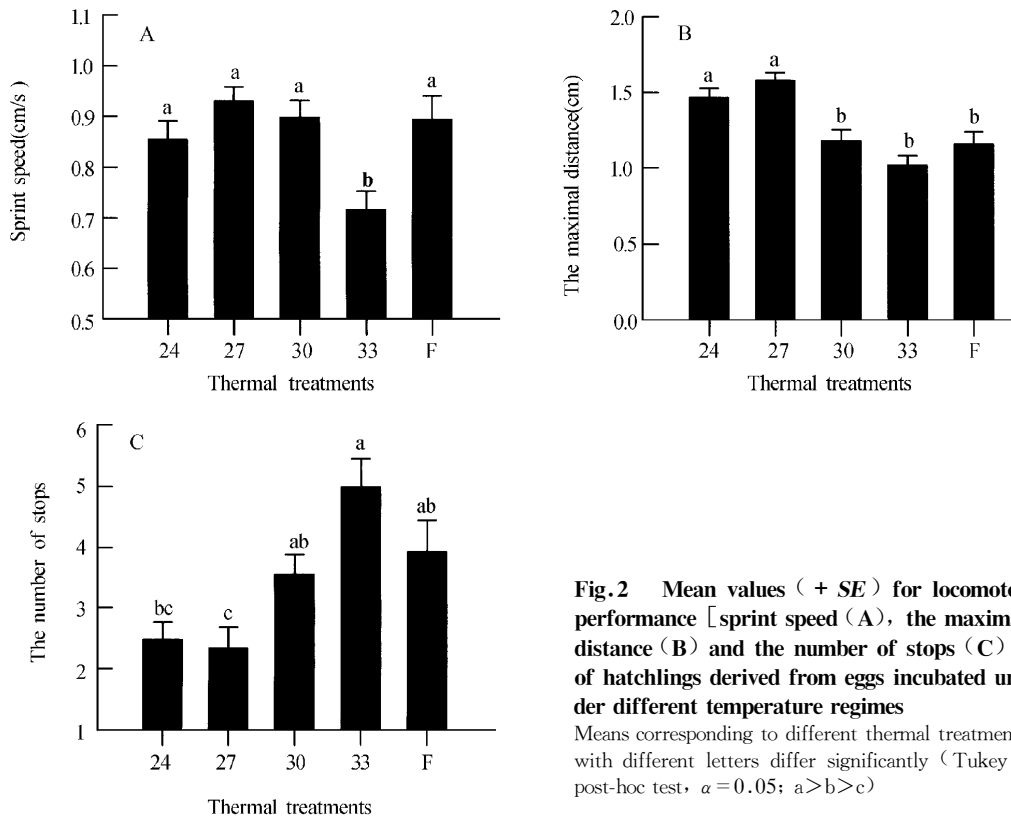
Fig. 2　Mean values（＋*SE*）for locomotor performance ［sprint speed（A），the maximal distance（B）and the number of stops（C）］ of hatchlings derived from eggs incubated under different temperature regimes

Means corresponding to different thermal treatments with different letters differ significantly（Tukey's post-hoc test，$\alpha = 0.05$；a＞b＞c）

3　Discussion

　　It has been documented in reptiles that high temperatures influence developing embryos differently than low temperatures. For example，exposure of reptilian eggs to extremely high temperatures markedly increases embryonic mortality and abnormality，whereas low temperatures，although slow or arrest embryonic development，usually have no lethal effect on embryos（Sexton and Marion，1974；Andrews and Rose，1994；Andrews et al.，1997）. The lower and upper threshold temperatures over which hatching success decreases dramatically differ not only among but also within species differing in habitat use and/or distribution. For example，eggs cannot be incubated at temperatures higher than 28℃ in *Scincella modesta*（slender forest skink）using cool habitats（Lu et al.，2006），whereas the detrimental effects on hatching success cannot be detected until eggs are incubated at temperatures higher than 30℃ in lizards such as *Takydromus septentrionalis*（northern grass lizard；Lin and Ji，1998），*T. wolteri*（white-striped grass lizard；Pan and Ji.，2001）and *Calotes versicolor*（oriental garden lizard；Ji et al.，2002b）using warm habitats. In *Eumeces chinensis*（Chinese skink），eggs from a lower latitudinal population have a narrower range of lower and upper threshold incubation temperatures than do those from a higher latitudinal population，primarily because of more stable thermal environments in the former population（Ji and Zhang，2001；Ji et al.，2002a）.

　　Thermal environments in northern China are characterized by the low mean but the great amplitude of thermal fluctuations. In face of these thermal environments，the extent to which lizards may enjoy reproductive benefits should depend on how well their eggs can tolerate extreme temperatures. Compared with the results reported for lizards living in warmer and thermally more stable regions（Lin and Ji，1998；Ji and Braña，1999；Pan and Ji，2001；Ji and Zhang，2001；Ji et al.，2002b），hatching successes at 24℃ and 33℃ are both high in *E. argus*，suggesting the existence of a widened range of viable incubation temperatures in lizards living in thermally more variable regions（Ji et al.，2002a）.

　　In this study，eggs incubated at fluctuating temperatures，depending on oviposition date，had the experience with being exposed to temperatures up to 36.1 – 37.7℃ occurring mainly between 1 200 – 1 400 h（Beijing time）. Prolonged exposure of eggs to temperatures higher than 33℃ has a lethal effect on embryos in all species of lizards studied to date，including *T. septentrionalis*（Lin and Ji，1998；Du and Ji，2006），*T. wolteri*（Pan and Ji.，2001），*E. chinensis*（Ji and Zhang，2001；Chen et al.，2003），*E. elegans*（blue-tailed skink；Du et al.，2003）and *C. versicolor*（Ji et al.，2002b）. Interestingly，however，the F-treatment did not differ significantly from the other four treatments in hatching success. This finding suggests that，as in other

species of reptiles（e.g., Sexton and Marion，1974；Andrews and Rose，1994；Andrews et al.，1997；Chen and Ji，2002；Chen et al.，2003；Du and Ji，2003；Ji et al.，2003；Du and Ji，2006），daily exposure of eggs to extreme temperatures for brief periods may not necessarily increase embryonic mortality in *E.argus*.

Incubation length decreases as incubation temperature increases in *E.argus*. This pattern is widespread in reptiles，although incubation length at any given temperature may differ among species differing in egg size and/or embryonic stage at oviposition. It is worthy noting，however，that a substantial portion（～88%）of variation in incubation length could be explained by the mean incubation temperature in the F-treatment and that the mean incubation length at any given mean incubation temperature was longer in this treatment（Fig.2）. These results suggest that，as in *Apalone mutica*（smooth soft-shelled turtle；Ashmore and Janzen，2003），thermal fluctuations may increase incubation length in *E.argus*. In other species of reptiles，however，thermal fluctuations either reduce incubation length（Overall，1994；Shine and Harlow，1996）or do not influence incubation length differently than constant temperatures with nearly the same mean（Georges et al.，1994；Andrews et al.，2000；Webb et al.，2001）. It seems that eggs of different reptilian species respond differentially to thermal fluctuations.

In this study，except for the effects of incubation temperature on body dry mass，residual yolk dry mass，carcass dry mass and the number of ventral scales，little variation was detected among measures of size and morphology of hatchlings across the five temperature treatments（Table 3）. Body dry mass and carcass dry mass differed among treatments，but the differences were actually very slight（Table 3）. Therefore，our results are largely consistent with the findings from similar studies of reptiles that show the existence of a range of temperatures within which no differential effects of incubation temperature on hatchling phenotypes can be detected（e.g.，Van Damme et al.，1992；Ji and Braña，1999；Braña and Ji，2000；Ji and Du，2001a，b；Ji and Zhang，2001；Ji et al.，2002b；Lin and Ji，2004）. The result that more yolks remained unutilized at hatching when eggs are incubated at high temperatures is not surprising，because it seems to be common in all species of reptiles studied to date（e.g.，Beuchat，1988；Phillips et al.，1990；Phillips and Packard，1994；Ji and Braña，1999；Ji and Du，2001a，b；Ji and Zhang，2001；Ji et al.，2002b；Lin and Ji，2004；Lin et al.，2005）.

Incubation temperatures within the range of 24－30℃ almost exerted no differential effects on size and morphology of hatchling in *E.argus*. Interestingly，however，incubation temperatures within the range significantly affected locomotor performance（both the maximal distance and the number of stops）of hatchlings，with hatchlings incubated at 24℃ and 28℃ performing better than did hatchlings incubated at 30℃（Fig.3）. This result provides strong evidence showing that hatchlings incubated at temperatures higher than 30℃ may exhibit major detrimental effects. Overall，locomotor performance was best in the low temperature treatments（24℃ and 27℃）and worst in the high temperature treatment（33℃），with the moderate temperature treatments（30℃ and F）in between. Unlike *A.mutica*（Ashmore and Janzen，2003）and *T.septentrionalis*（Du and Ji，2006）in which increased thermal variance during embryonic development leads to enhanced locomotor performance of hatchlings，thermal fluctuations did not positively affects locomotor performance of hatchlings in this study. However，whether this difference can be attributed to differential embryonic responses to thermal fluctuations in different reptilian species or to extremely high temperatures（>36℃）experienced by developing *E.argus* embryos remains unknown.

Taken together，our data show that daily exposure of eggs to extreme temperatures that are potentially lethal to embryos for brief periods does not have detectable adverse effects on hatching success and morphological phenotypes of hatchlings in *E.argus*. Of the hatchling phenotypes examined，body dry mass，carcass dry mass，residual yolk dry mass and locomotor performance are more likely to be affected by incubation temperature. Thermal fluctuations exert no positive effects on locomotor performance of hatchlings for some unknown reasons，but influence incubation length differently than constant temperatures with the same mean.

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