Temperature acclimation affects thermal preference and tolerance in three Eremias lizards Lacertidae

Hong Li1 Zheng WANG1 Wenbin MEI1 Xiang Ji1,2 *

1. Jiangsu Key Laboratory for Biodiversity and Biotechnology College of Life Sciences Nanjing Normal University Nanjing Jiangsu 210046 China
2. School of Life Sciences Hangzhou Normal University Hangzhou Zhejiang 310036 China
3. Department of Biological Sciences Central Washington University Ellensburg WA 98926-7537 USA

Abstract We acclimated adult males of three Eremias lizards from different latitudes to 28°C, 33°C, or 38°C to examine whether temperature acclimation affects their thermal preference and tolerance and whether thermal preference and tolerance of these lizards correspond with their latitudinal distributions. Overall selected body temperature Tsel and viable temperature range VTR were both highest in E. brennelyi and lowest in E. multisocellata with E. argus in between. Critical thermal minimum CTMin was highest in E. multisocellata and lowest in E. brennelyi with E. argus in between. Critical thermal maximum CTMax was lower in E. multisocellata than in other two species. Lizards acclimated to 28°C and 38°C overall selected lower body temperatures than those acclimated to 33°C. Lizards acclimated to high temperatures were less tolerant of low temperatures and vice versa. Lizards acclimated to 28°C were less tolerant of high temperatures but had a wider VTR range than those acclimated to 33°C and 38°C. Lizards of three species acclimated to the three temperatures always differed from each other in CTMin but not in Tsel CTMax and VTR. Our results show that temperature acclimation plays an important role in influencing thermal preference and tolerance in the three Eremias lizards although the degrees to which acclimation temperature affects thermal preference and tolerance differ among species thermal preference rather than tolerance of the three Eremias lizards corresponds with their latitudinal distributions. Current Zoology 55 4 258 – 265 2009.

Key words Lizards Eremias Acclimation temperature Thermal preference Thermal tolerance Viable temperature range Food assimilation

As ectotherms lizards can withstand a wide range of body temperatures. However extremely low or high temperatures may certainly lead them to death. The upper critical thermal maximum CTMax and lower critical thermal minimum CTMin survival limits are often defined as the upper and lower extremes of thermal tolerance at which the animal cannot right itself when placed on its back. e. g. the loss of righting response Cowles and Bogert 1944 Lowe and Vance 1955 Brown and Feldmeth 1971 Doughty 1994 Lutterschmidt and Hutchison 1997. Many lizards attempt to maintain relatively high and constant body temperatures when conditions allow them to do so often because biochemical and physiological activities are maximized at moderate to relatively high temperatures Huey and Stevenson 1979 Huey 1982 Huey and Kingsolver 1989 Kauffman and Bennett 1989 Angillette 2001 Angillette et al. 2002. Lizards acquire and maintain appropriate body temperatures mainly through behavioral mechanisms although physiological thermoregulation through cardiovascular adjustments endogenous heat production and evaporative cooling may be also important Avery 1982.

Bartholomew 1977 1982 Huey 1982 1991. The body temperature preferred by a lizard often corresponds closely to the optimal temperature range for biochemical and physiological activities and can be estimated by measuring its selected body temperature Tsel in a laboratory thermal gradient Angillette et al. 2002 and references therein.

Thermal preference Tsel and tolerance CTMin and CTMax have been studied in diverse animal taxa. Studies of lizards have shown that thermal preference and tolerance may vary among and within species as a response to changes in the thermal environment associated with habitat use or geographic distribution Huey and Kingsolver 1993 Feder et al. 2000 Angillette et al. 2002 Winne and Keck 2005 Du 2006 and among individuals of the same population that differ in physiological or developmental conditions Hutchison 1976 Daut and Andrews 1993 Mathies and Andrews 1997 Le Galliard et al. 2003 Du et al. 2008 Lin et al. 2008. For example lizards from cool habitats usually select lower body temperatures than do those from warm habitats Xu and Ji 2006 and references therein and females usually shift thermal preference when
pregnant due to behavioral preferences or due to constraints on thermoregulation activities\[ Braña\[ 1993\] Mathies and Andrews\[ 1997\] Le Galliard et al.\[ 2003\] Ji et al.\[ 2006\] Lin et al.\[ 2008\].

Thermal preference and tolerance are subject to phenotypic alteration within limits that are genetically fixed. Phenotypic plasticity depends on a variety of factors the principal of which is thermal acclimation\[ a process by which organisms physiologically adjust to an altered thermal environment \[ Patterson and Davies\[ 1978\] Cossins and Bowles\[ 1987\] Lutterschmidt and Hutchison\[ 1997\] Andrews\[ 1998\] Rock et al.\[ 2000\] Brown and Griffin\[ 2005\]. Thus\[ determination of thermal preference and tolerance based on field observations may not always depict true preference and tolerance. Therefore\[ thermal preference and tolerance have usually been measured in the laboratory where animals are maintained under strictly controlled thermal conditions.

Here\[ we present data on thermal preference and tolerance of three species of lacertid lizards\[ Eremias argus\[ E. brenchleyi and E. multiocellata\] acclimated to three temperatures\[ see below for details\]. We addressed the following questions\[ Can temperature acclimation alter thermal preference and tolerance in the lizards studied\[ Do these lizards show interspecific differences in thermal preference and tolerance when acclimated under identical thermal conditions\[ If so\] do any such differences correspond with their latitudinal distributions\[?

1 Materials and Methods

1.1 Study animals

The three congenic species are all small-sized lizards with an exclusively temperate distribution. *Eremias argus*\[ Mongolian racerunner\] is an oviparous lizard that ranges from northern China\[ southward to Jiangsu and westward to Qinghai\] to Russia\[ region of Lake Baikal\] Mongolia and Korea\[ Zhao\[ 1999\].

*Eremias brenchleyi*\[ Ordos racerunner\] is an oviparous lizard that is endemic to China and lives in several eastern and northern provinces of the country\[ Chen\[ 1991\].

*Eremias multiocellata*\[ multi-ocellated racerunner\] is a viviparous lizard that ranges from northern China\[ southward to Gansu and eastward to Liaoning\] to Mongolia\[ Kirgizstan\] Kazakstan and Russia\[ Tuvin District in Siberia\] Zhao\[ 1999\].

We collected adults of *E. argus* from Linfen\[ 36° 06’ N 111° 33’ E\] Shanxi\[ northern China\] in mid-April 2007\[ E. brenchleyi from Suzhou\[ 33° 38’ N 116° 59’ E\] Anhui\[ eastern China\] in early April 2007\[ and *E. multiocellata* from Wulatetouqi\[ 41° 27’ N 106° 59’ E\] Inner Mongolia\[ northern China\] in mid-May 2007.

Fig.1 shows monthly variation in air temperatures of the three localities. Lizards were transported to our laboratory at Hangzhou Normal University\[ where they were marked by a non-toxic waterproof label for future identification. Eight to 12 lizards of the same species were housed in a 750 mm × 500 mm × 450 mm\[ length × width × height\] plastic cage that contained a substrate of sand\[ with rocks and pieces of clay tiles provided as cover. Cages were placed in a room kept at 22 ± 2°C. A 100-W light bulb suspended at one end of each cage created a thermal gradient from the room temperature to 55°C for 14 h daily. Lizards were exposed to a natural light cycle\[ and could thermoregulate during the photophase. Lizards were fed mealworms\[ larvae of *Tenebrio molitor*\] house crickets *Acheta domesticus* and water enriched with vitamins and minerals *ad libitum*. Females laid eggs

![Monthly mean ± SE air temperatures for 1987 – 2007 at the three localities courtesy of the Provincial Bureaus of Meteorology of Anhui, Shanxi and Inner Mongolia](image-url)

Fig.1 Monthly mean ± SE air temperatures for 1987 – 2007 at the three localities courtesy of the Provincial Bureaus of Meteorology of Anhui, Shanxi and Inner Mongolia\[ where lizards were collected\].
E. argus and E. brenchleyi or gave birth to young E. multiocellata under the laboratory conditions described above.

1.2 Thermal acclimation of animals

In September when lizards had been kept under identical laboratory conditions for about 4–5 months we selected 36 E. argus 2.8 – 4.7 g and 36 E. brenchleyi 3.3 – 5.8 g and 36 E. multiocellata 3.6 – 6.4 g males with an intact tail to conduct acclimation experiments. Lizards of each species were equally divided into three groups each of which was assigned to one of the three temperature treatments 28 33 and 38 °C. These three temperatures were chosen because lizards of two E. argus and E. brenchleyi of the three species had been known to feed normally at temperatures within the range of 28 – 38 °C Luo et al. 2006 Xu and Ji 2006. Lizards individually housed in 300 mm x 250 mm x 300 mm glass cages were acclimated for three weeks at their designated temperatures followed by Tsel CTMin and CTMax measurements. During this period lizards did not have any heat source for baking and based on the results reported for E. argus Luo et al. 2006 and E. brenchleyi Xu and Ji 2006 the body temperatures of the test groups and very close to 28 °C 33 °C or 38 °C.

Feces urates and subsamples of food mealworms corresponding to each lizard were dried to constant mass at 65 °C and weighed. Dried samples were burnt in a WGR-1 adiabatic calorimeter Changsha Instrument China and data on energy density were automatically downloaded to a computer. The assimilation efficiency AE was calculated as 100 × I – F – U / F where I = energy ingested F = energy in feces and U = energy in urates. The apparent digestive coefficient ADC was calculated as 100 × I – F / I. 1.3 Determination of Tsel CTMin and CTMax

We measured Tsel in 1000 mm x 800 mm x 500 mm plastic cages with 5 cm depth sand and pieces of clay tiles. A 100-W light bulb suspended above one end of the terrarium created a thermal gradient from the room temperature 22 °C to 55 °C for 14 h daily. Lizards were individually introduced into the gradient at the cold end at 06:00 Beijing time when the lights were switched on. To minimize the potential influence of diel variation in Tsel we began measurements every trial day at 15:00 and ended within two hours. Both Tsel cloacal temperatures to the nearest 0.1 °C were taken for each lizard that was basking on the surface with a UT325 digital thermometer Shanghai Medical Instruments China. The probe ~1 mm diameter was inserted ~4 mm into the cloaca when used to measure a lizard’s body temperature and great care was taken to avoid heat transfer between the hand and the lizard. To address the repeatability of our measurements we measured each lizard five times once on each of five consecutive days. The five measures did not differ significantly within each species × temperature combination repeated measures ANOVA all P > 0.216. We therefore considered the mean of the five measures as a lizard’s Tsel. During the intervals of Tsel trials lizards were put back to their house cages before CTMin and CTMax were measured.

We used FPQ incubators Ningbo Life Instruments China to determine CTMin and CTMax. Trials were conducted between 10°00 – 15°00. We cooled or heated lizards from their designated acclimation temperatures at a rate of 0.25 °C min−1 and at a slower rate of 0.1 °C min−1 when temperatures inside the incubator were lower than 12 °C or higher than 40 °C. During the trials we observed the behavior of lizards through a window in the incubator door. Lizards were taken out of the incubator for the righting response test and body temperatures associated with a transient loss of the righting response at the lower and the upper limits of thermal tolerance were considered to be the endpoints for CTMin and CTMax respectively.

1.4 Statistical analyses

We used Statistica software package version 5.0 for PC to analyze the data. Body mass was not a significant predictor of all examined traits within each species × temperature combination all P > 0.229. We therefore used two-way ANOVA to examine the effects of species temperature acclimation and their interaction on the examined traits combined with Tukey’s post hoc test. Percentage data on ADC and AE were arcsine-transformed prior to parametric analyses. One-way ANOVA was used to examine whether lizards acclimated to any particular temperature showed interspecific differences in thermal preference and tolerance. All values are presented as mean ± SE and the significance level is set at α = 0.05.

2 Results

Data for the effects of body temperature on food intake AE and ADC are given in Fig. 2. Food intake AE and ADC differed among species and among temperature treatments food intake and ADC were affected by the species × temperature interaction whereas AE was not Table 1. Food intake was greatest in E. brenchleyi and smallest in E. multiocellata with E. argus in between lizards at 33 °C and 38 °C did not differ in food intake but they both ingested more food than did those at 28 °C Table 1. AE and ADE were both higher in E. brenchleyi than in other two species AE and ADC were both highest at 38 °C and smallest at 28 °C with the 33 °C treatment in between Table 1.

Data for the effects of acclimation on thermal preference and tolerance are given in Fig. 3. Tsel CTMin CTMax and viable temperature range the difference between CTMax and CTMin VTR differed among species and among temperature treatments Tsel
CTMin and CTMax were all affected by the species × temperature interaction\(^*\) whereas VTR was not\(^*\) Table 1\(^*\). Tsel was highest in *E. brengleleyi* and lowest in *E. multiocellata* with *E. argus* in between\(^*\) lizards acclimated to 28°C and 38°C overall selected lower body temperatures than did those acclimated to 33°C\(^*\) Table 1\(^*\). CTMin was highest in *E. multiocellata* and lowest in *E. brengleleyi* with *E. argus* in between\(^*\) lizards acclimated to high temperatures were less tolerant of low temperatures\(^*\) and vice versa\(^*\) Table 1\(^*\). *Eremias argus* and *E. brengleleyi* were overall more tolerant of high temperatures than *E. multiocellata*\(^*\) lizards acclimated to 28°C were less tolerant of high temperatures than those acclimated to other two higher temperatures\(^*\) Table 1\(^*\). VTR was greatest in *E. brengleleyi* and smallest in *E. multiocellata*\(^*\) with *E. argus* in between\(^*\) VTR was greater in the 28°C treatment than in other two treatments\(^*\) Table 1\(^*\).

Lizards of three species acclimated to the three temperatures always differed from each other in CTMin\(^*\)
but not in Tsel, CTMax and VTR. Within each temperature treatment, CTMin was highest in *E. multiocellata* and lowest in *E. brenchleyi* with *E. argus* in between. Tukey’s test all $P < 0.013$. The three *Eremias* lizards did not differ in Tsel in the 38°C treatment One-Way ANOVA $F_{2,13} = 0.84$ $P = 0.442$ and in no temperature treatment did Tsel differ between *E. argus* and *E. brenchleyi* Tukey’s test all $P > 0.060$. CTMax did not differ between *E. argus* and *E. brenchleyi* in the 28°C treatment Tukey’s test $P = 0.273$ nor between *E. argus* and *E. multiocellata* in the 38°C treatment Tukey’s test $P = 0.742$. VTR did not differ between *E. argus* and *E. brenchleyi* in the 33°C treatment Tukey’s test $P = 0.238$. Thus, whereas two-way ANOVA overall revealed that thermal preference and tolerance differed among species the three *Eremias* lizards did not always show interspecific differences in thermal preference and tolerance when acclimated under identical thermal conditions. Xu and Ji 2006 and references therein. Consistent with the many previous studies of lizards e.g. Lowe and Vance 1955 Brattstrom 1971 Patterson 1999 Huang et al. 2006 Yang et al. 2008 temperature acclimation had significant impacts on thermal preference and tolerance in the three *Eremias* lizards.

Thermal preference was more likely to be affected by acclimation temperature in *E. brenchleyi* and *E. multiocellata* than in *E. argus* suggesting that the degree to which thermal preference is affected by acclimation temperature differs among lizard species. The three *Eremias* lizards all had an ample opportunity to maintain their body temperatures at any level in the laboratory thermal gradient. Interestingly however in no species was Tsel maximized in individuals acclimated to the highest temperature Fig. 3. The explanation for this result presumably lies in the trade-off between costs and benefits associated with thermoregulation. Thermoregulation may result in potential fitness benefits but the benefits are offset by any costs associated with thermoregulation in any given set of environmental conditions Shine and Madsen 1996 Sartorius et al. 2002 Lin et al. 2008. Thus lizards should shift their thermal preferences according to changes in thermal

**Fig. 3** Mean values $\pm SE$ for selected body temperature, critical thermal minimum, critical thermal maximum, and viable temperature range of lizards of three species acclimated to different temperatures.
environment towards the levels optimal for physiological or behavioral performances and minimize costs associated with thermoregulation to a large extent.

Hertz et al. 1993 Christian and Weavers 1996 Blouin-Demers et al. 2000 Angilletta et al. 2002 Lin et al. 2008. In many lizards including two Eremias lizards E. argus and E. brenchleyi body temperatures varying over a relatively wide range do not have statistically differential effects on several important performances such as food intake food assimilation and locomotion but energetic demands do increase with an increase in body temperature Yang et al. 2008 and references therein. Thus the result that lizards did not select higher-than-usual body temperatures when acclimated to the highest temperature could reflect a mechanism evolved in the three Eremias lizards to reduce energetic costs associated with the increased metabolic rates at high body temperatures.

Lizards acclimated to low temperatures were more tolerant of low temperatures than those acclimated to high temperatures and vice versa Fig. 3. Such a pattern is shared by the three Eremias lizards and has been reported for other lizards such as the south China forest skink Sphenomorphus incognitus Huang et al. 2006 the Taiwan forest skink S. taiwanensis Huang et al. 2006 and the northern grass lizard Takydromus septentrionalis Yang et al. 2008. The upper limit of thermal tolerance in general increased with an increase in acclimation temperature in E. brenchleyi and E. multiocellata Fig. 3. This pattern also occurs in S. incognitus S. taiwanensis and T. septentrionalis Huang et al. 2006 Yang et al. 2008. In E. argus however individuals acclimated to a medium temperature 33°C were the most tolerant of high temperatures Fig. 3. This difference is of interest because it suggests that CTMax may not always be maximized at the highest acclimation temperature in lizards.

Means of Tsel CTMin and CTMax for adult E. argus from the same area measured in April monthly mean air temperature is ~14°C are 36.0 ± 1.0 and 44.9 ± C respectively Luo et al. 2006 means of Tsel CTMin and CTMax for adult E. brenchleyi from the same area measured in April monthly mean air temperature is ~18°C are 33.5 ± 3.4 and 43.6 ± C respectively Xu and Ji 2006. In both species means of Tsel and CTMax for the field-caught individuals fall within the ranges of Tsel 33.6 – 36.7°C in E. argus 34.0 – 38.4°C in E. brenchleyi and CTMax 41.6 – 44.7°C in E. argus 41.1 – 45.2°C in E. brenchleyi for individuals acclimated to 28°C the lowest acclimation temperature in this study. Nonetheless means of CTMin are much lower in the field-caught individuals than in those 6.5 – 9.5°C in E. argus with a mean of 8.0°C 4.3 – 5.8°C in E. brenchleyi with a mean of 4.3°C acclimated to 28°C. These results are consistent with an earlier study of T. septentrionalis that has found acclimation temperature to have a more significant impact on CTMin than on CTMax or Tsel Yang et al. 2008.

Thermal environments change dramatically with latitude or altitude with mean air temperature in general decreasing with an increase in latitude or altitude. In mainland China northern high latitudinal areas are characterized by a low mean but great amplitude of thermal fluctuations that may be an important factor restricting basking opportunities for ectotherms including lizards. Thus intuitively lizards with a northerly distribution should be more tolerant of extreme temperatures and select lower body temperatures than those with a southerly distribution. Interestingly however E. multiocellata had higher CTMin but lower CTMax than other two congenic species E. argus and E. brenchleyi with more southerly distributions and CTMin was higher in E. argus than in E. brenchleyi with a more southerly distribution Table 1. Thus inconsistent with many previous studies of lizards that have found a clear relation between thermal tolerance and latitudinal or altitudinal distributions Spellerberg 1972 Hertz et al. 1979 Hertz 1981 Wilson and Echternacht 1987 Lemos-Espinal and Ballinger 1995 Huang et al. 2006 the results of this study show that thermal tolerance of the three Eremias lizards does not correspond with their latitudinal distributions. Similar results i.e. lacking correlations between thermal tolerance and geographic distributions have been also reported for several lizard species such as the starred agama Stellio stellatus Hertz and Nevo 1981 the eastern fence lizard Sceloporus undulatus Crowley 1985 the striped skink Mabuya striata Patterson 1999 and T. septentrionalis Yang et al. 2008.

Correlations between thermal preference and latitudinal or altitudinal distributions could not be easily detected in many lizards often because thermal preference is highly associated with their use of habitats rather than geographic distributions. For example lizards such as the brown forest skink Sphenomorphus indicus 25.7°C Ji et al. 1997 using shaded and thus cold habitats select lower body temperatures than those such as the Chinese skink Euneces chinesis 31.2°C Ji et al. 1995 living in the same area but using exposed and thus warm habitats. The three Eremias lizards studied all use exposed habitats in nature but differ from each other in thermal preference with Tsel being lower in higher latitudinal species than in lower latitudinal species Table 1. This finding provides clear evidence showing that thermal preference of the three species corresponds with their latitudinal distributions.

Collectively our results show that temperature acclimation plays an important role in influencing thermal preference and tolerance in three congeners of Eremias
although the degrees to which thermal preference and tolerance are altered by acclimation temperature differ among species, Tsel CTMin CTMax and VTR overall differ among the three *Eremias* lizards but these lizards always differ from each other in CTMin but not in other three variables when acclimated under identical thermal conditions. Our results also show that thermal preference rather than tolerance of the three *Eremias* lizards corresponds with their latitudinal distributions.

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**References**


