

Chapter 1

General Introduction

Every species has its own geographical distribution. Understanding the factors that determine a species' distribution is a central objective in ecology (e.g. Caughley et al. 1988, Lawton et al. 1994, Brown et al. 1996, Brown and Lomolino 1998, Krebs 2001) and evolutionary biology (Jablonski 1987). It also provides important information for further applications in predicting species distributions in response to environmental change (Holt 2003) and species conservation (Wikelski and Cooke 2006).

The distribution of a species is influenced by its dispersal limitations, biotic interactions (i.e. competition, predators, parasitism, disease, etc.), and environmental suitability (i.e. temperature, humidity, light, etc.) (Krebs 2001). The role of environmental factors is acknowledged to have the potential to affect the geographic distribution range and abundance of a species (Dobzhansky 1950, MacArthur 1972).

Among the environmental factors, temperature is particularly important (Stuart, 1951; Graham et al., 1971; Greer, 1980; Huang et al., 2006) in affecting ectotherms' geographic distributions. This is because its impact on the body temperatures of a species (Brattstrom, 1965) often influences physiological functions and behavioral performance (Bennett, 1980; Kaufmann and Bennett, 1989; Angilletta, 2001).

In this dissertation, I aimed to figure out the environmental factors that affected the altitudinal distributions of *Takydromus* lizards in Taiwan. There are five *Takydromus* species living in different altitudes on Taiwan island (Lue et al., 1999): *T. hsuehshanensis* (> 1800m), *T. formosanus* (< 1500m), *T. stejnegeri* (<1000m), *T. sauteri* (<1000m), and *T. kuehnei*, <1000m). Recently, two cryptic species (*T. viridipunctatus*, *T. luyeanus*), previously confused with *T. formosanus*, were indentified using mitochondrial DNA

sequences (Lue and Lin, 2008). Because of being limited by the amount of work I could handle, I only used three species distributed along different altitudinal ranges to study. These three species were *T. hsuehshanensis*, *T. viridipunctatus*, and *T. stejnegeri*. I chose lowland *T. stejnegeri* instead of the other two lowland species (*T. sauteri*, and *T. kuehnei*) because it was more closely- related to the high altitude *T. hsuehshanensis*. As for mid-altitudinal species, since the relationship of *T. viridipunctatus*, *T. formosanus* and *T. luyeanus* had the same close relationship to *T. hsuehshanensis* (Lue and Lin, 2008), there was no difference in consideration of phylogenetic closeness. I therefore chose *T. viridipunctatus* for its relatively easy collecting in the wild.

This study had two main objectives: one was to investigate the difference in thermal physiology and its role on altitudinal distributions of these three species, and the other was to investigate whether the mountainous *T. hsuehshanensis* was able to live well under the lowland environment. To achieve the first objective, I analyzed three thermal physiological traits of these three species: the heat thermal tolerance, the cold thermal tolerance, and the sprint speed. All of these were reported to have a great impact on animals' survival (Christian and Tracy 1981, Huang et al. 2006). To achieve the second objective, I conducted a transplant experiment on the mountainous *T. hsuehshanensis*. I moved it to semi-natural outdoor enclosures in lowland areas and recorded its growth and survival status during a one year period.

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Chapter 2
**Heat tolerance and altitudinal distribution of three *Takydromus* lizards
in Taiwan**

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INTRODUCTION

Environmental factors, such as temperature, humidity, and oxygen content, have important influences on the geographical distributions of ectotherms (Krebs, 1994). Among these factors, temperature is particularly important (Stuart, 1951; Graham et al., 1971; Greer, 1980; Huang et al., 2006) because of its impact on the body temperatures of ectotherms (Brattstrom, 1965), which often influences physiological functions and behavioral performance (Bennett, 1980; Kaufmann and Bennett, 1989; Angilletta, 2001). Ectotherms frequently do not thermoregulate metabolically as endotherms do, and therefore must select between appropriate thermal microhabitats to maintain their optimal body temperatures (Brattstrom, 1965, 1970a, 1979; Spellerberg, 1972a; Huey and Pianka, 1977; Hertz and Huey, 1981; Avery, 1982; Huey, 1982). Although ectotherms are capable of behaviorally regulating their body temperatures, their physical environments eventually limit the extent of their thermoregulation (Huey, 1974; Huey and Stevenson, 1979; Wu and Kam, 2005). I therefore expected that environmental temperatures might affect the survival of ectotherms and their geographic distribution ranges.

Because thermal environments change dramatically with altitude, with mean temperature decreasing at a rate of 0.6 °C for every 100 meters increase in altitude (Bouverot, 1985), ectothermic vertebrates living at high altitudes experience lower environmental temperatures than do their lowland closely-related counterparts. Some

studies have shown that high altitude ectotherms, as expected, adapt to lower ambient temperatures by physiological or behavioural means (Stuart, 1951; Hertz et al., 1979; Hertz, 1981; Hillyard, 1981; Navas, 1997). On the other hand, compared to their lowland relatives, high altitude ectotherms might be less heat tolerant, especially for those animals which are not able to accurately regulate their body temperature behaviourally, such as most amphibian species (Delson and Whitford, 1973; Miller and Packard, 1974, 1977; Hoppe, 1978), and some reptile species (Hertz et al., 1979; Hertz, 1981; Huang et al., 2006).

High temperatures occurring in areas beyond the native range of mountain reptiles might be an important limiting factor for current altitudinal distributions. However, I found that most previous studies in this area frequently used critical thermal maximum (CTMax) to measure ectotherms' heat tolerance capacity (Brattstrom, 1965; Spellerberg, 1972a, b; Hertz et al., 1979; Hertz, 1981). The CTMax is an estimator of acute heat tolerance, defined as "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death" (Cowles and Bogert, 1944). Accordingly, this measure is directly related to the ecology of a species (Lowe and Vance, 1955; Hutchison, 1961). However, since some harmful physiological effects may arise only after prolonged exposure to a specific temperature (Cowles and Bogert, 1944; Licht, 1965, 1971; Huang et al., 2006), the examination of chronic thermal tolerance is needed to achieve a complete understanding of heat tolerance. For example, in our previous study (Huang et al., 2006), we found that although the CTMax of a high mountainous lizard (*S. taiwanensis*) reached 38.6 °C, which is high enough for it to endure normal high temperature in lowland area for a short

period of time, it could not survive well at 30 °C (approximately the mean temperature in lowland area) for a long period of time.

The aim of this study was to evaluate whether the heat tolerance of an endemic grass lizard living at altitudes above 1,800 m, *Takydromus hsuehshanensis*, is a biological factor that prevents them from migrating to low altitudes. I examined and compared both its CTMax and chronic heat tolerance (survival rate and body mass) to that of two of its lowland congeners, *T. formosanus* (currently revised as *T. viridipunctatus*, Lue and Lin, 2008) (< 1,500 m altitude) and *T. stejnegeri* (< 1000 m altitude) (Lue et al., 1999). Since CTMax may change with acclimation temperatures (Brattstrom and Regal, 1965, Brattstrom, 1970b, 1971; Hutchison and Rowland, 1974), it was measured after 2 weeks of acclimation period at three temperature treatments (10°C, 20 °C, and 30 °C). For chronic heat tolerance, I measured 3-month survival rates and body mass changes under 3 fluctuating daily temperature treatments. Two of these treatments were set to approximating lowland summer temperature recordings (extremely high temperature and average temperature, respectively) and another was set to approximating ambient temperatures in mountain areas. I predicted that: (1) CTMax of *T. hsuehshanensis* would be lower than the maximal ambient temperature in lowland areas and its CTMax would be the lowest among the three species, (2) *T. hsuehshanensis* could not survive under extreme high temperature and/or average high temperature treatments over a 3 month period, but the lowland *T. stejnegeri* and *T. formosanus* would survive well under these two treatments, and (3) CTMax would increase with the elevated acclimation temperatures.

MATERIALS AND METHODS

Animal collection and maintenance

From May to July 2004, I collected male lizards for CTMax measurement. I captured *T. hsuehshanensis* (n = 39, SVL \pm 1SE = 58.5 ± 1.1 mm, mass \pm 1SE = 4.2 ± 0.2 g) from Mt. Hehuan (2,400 – 3,200 m in altitude, Nantou County); *T. stejnegeri* (n = 49; SVL \pm 1SE = 50.6 ± 0.6 mm, mass \pm 1SE = 2.5 ± 0.06 g) from Taoyuan and Taipei counties (approximately sea level), and *T. formosanus* (n = 30; SVL \pm 1SE = 48.9 ± 0.6 mm, mass \pm 1SE = 2.3 ± 0.09 g) from Taipei county (approximately sea level), Taiwan. From May to August 2005, I collected each species from the same locations for the chronic heat tolerance experiment. I captured *T. hsuehshanensis* (n = 50, male: SVL \pm 1SE = 60.1 ± 0.7 mm, mass = 5.0 ± 0.2 g, n = 25; female: SVL \pm 1SE = 60.8 ± 0.7 mm, mass \pm 1SE = 4.5 ± 0.1 g, n = 25), *T. stejnegeri* (n = 51, male: SVL = 51.7 ± 0.9 mm, mass = 2.9 ± 0.1 g, n = 26; female: SVL = 54.4 ± 0.8 mm, mass = 3.1 ± 0.1 g, n = 25), and *T. formosanus* (n = 50, male: SVL = 52.7 ± 0.6 mm, mass \pm 1SE = 3.0 ± 0.1 g, n = 25; female: SVL \pm 1SE = 51.6 ± 0.4 mm, mass \pm 1SE = 2.6 ± 0.07 g, n = 25). After they were brought into the laboratory, all the animals were housed individually in plastic boxes (length \times width \times height = 20 cm \times 10 cm \times 15 cm) containing dry grass and a piece of tile as shelter. These boxes were placed in a laboratory (~ 25 °C) with photoperiod of L: D = 12hr: 12hr for less than 1 month before the experiment. The lizards were provided with water and food (crickets and mealworms dusted with vitamin powder) *ad lib*. I monitored their body mass as a health indicator, and lizards that lost over 10 % of their original body mass (n = 7) were excluded from all experiments.

Critical Thermal Maximum (CTMax) measurement

To prevent the potential confounding effects of gestation in females, I only used adult male lizards in the CTMax experiment. Individuals from each species were assigned randomly into 3 temperature groups (10, 20, or 30 °C). Each group for each species contained lizards of approximately the same mean body weight. CTMax was measured after a temperature acclimation treatment of two weeks with photoperiod L : D = 12hr : 12hr. Prior to CTMax measurement, animals were fasted for 48 hours.

I used both “lose righting reflex” (LRR) and “onset of spasm” (OS) as the behavioural criteria of CTMax (Brattstrom, 1968; Lutterschmidt and Hutchison, 1997a). The body temperature (T_b) of each tested lizard was measured by inserting a thermocouple probe (K type, 0.1 mm in diameter), connected to a data logger (RS-232, Thermolog 302, Center Technology Corp.) 1cm into the cloaca, and securing it to the tail with surgical tape. To measure CTMax, I set up an electric heater (1000 W) on a bench at room temperature (25 °C) in a laboratory. I raised the tested animals’ T_b gradually by 0.6 to 0.9 °C/ minute as recommended by Lutterschmidt and Hutchison (1997b). I set 3 plastic test boxes (length × width × high = 17 cm × 10 cm × 10 cm) to ~30 °C, ~40 °C, and ~50 °C by placing them on the bench at different distances from the electric heater (60 cm, 40 cm, and 20 cm respectively). I warmed the lizards by putting them sequentially in these boxes. I first placed a lizard in the 30 °C test box and recorded its T_b every 30 seconds. Once the increasing rate of its T_b fell below 0.3 °C during any 30 second period, I transferred it into the next test box with a higher temperature, and so forth. During this heating process, I noticed that when the test lizard’s T_b rose above ~38 °C, it tried to escape by climbing up the walls of the box. Thereafter, as the T_b continued

rising, the test lizard began to lose muscular coordination and failed to walk normally. As soon as it showed signs of losing muscular coordination, I started to check its righting reflex every 20 seconds. I recorded the lizard's Tb at which it lost its righting reflex as LRR. I usually observed muscle spasm on its forelegs within 2 minutes after recording LRR. Consequently, I recorded the Tb at which muscle spasms occurred as OS. Immediately after obtaining the OS, I placed the lizard into a box containing water at ~25 °C to cool it. Most of the lizards recovered within 5 minutes; however, 9 lizards died within 24 hours of the experiment, whose data were excluded from analysis.

Survival rate and body mass measurements

To design the experimental temperatures for the heat treatments, I analyzed the summer temperature recordings measured in 3 cities in the northern (Taipei), middle (Taichung), and southern (Kaoshiung) part of Taiwan (1897 - 1990, Central Weather Bureau, Taiwan). The historical maximum air temperature in these cities ranged from 37.8 - 39.3 °C, with daily fluctuating ranges normally from 8 - 11 °C. There were only 8 recordings above 38.0 °C. The means of daily maximum temperature, daily minimum temperature, and daily temperature were 32.4 °C, 25.2 °C, and 28.9 °C, respectively. Based on these records, I established 2 hot temperature treatments: the Extreme High (EH) temperature treatment and the High (H) temperature treatment. The EH treatment was set at a fluctuating daily range between 28 °C - 38 °C, with mean of 32.8 °C (Fig. 1), to approximate the historical maximum temperature range in the lowland area of Taiwan. The second H treatment was set at a fluctuating daily range between 26 °C - 33 °C, with mean temperature of 28.9 °C (Fig. 1), to approximate the general summer daily

temperature fluctuation in lowland areas. Besides these two treatments, I established a third cool (C) treatment. Its nocturnal temperature was set to 10.0 °C to approximate the night temperature during the summer in the high mountains (Mt. Alishan, 2,400 m in altitude, 1970 - 2000, Taiwan Central Weather Bureau) and its daily highest temperature was set to 32.0 °C to provide appropriate heat for the lizards' normal growth. These temperature treatments were conducted with three thermal incubators, whose programs were set at 8 - 9 different temperature intervals to fit the daily temperature fluctuations. Temperatures inside each thermal incubator were recorded with temperature data loggers (HOBO U12 thermometer, Onset Corporation, Bourne, MA), and they were very close to our proposed settings (Fig. 1).

Chronic heat tolerance measurements were taken during August - November 2005. In mid-August 2005, lizards from each species were randomly assigned into the 3 temperature treatments (EH, H, and C treatments). Each treatment contained lizards of approximately same mean weight and sex ratio. Lizards from each group were put individually into a plastic box (length × width × height = 17 cm × 10 cm × 10 cm), containing dry grass and a piece of tile as shelter. Afterwards, lizards of each group were placed into their assigned thermal incubators. They were fed crickets twice a week and provided with mealworms all the time. Water was provided *ad lib*. I measured their body weights and recorded the survival situation weekly.

Data analysis

I used a two-way multivariate analysis of variance (MANOVA) to estimate the effects of species, acclimation temperatures, and interactions on two CTMax measures

(NRR and OS). A Scheffé post hoc comparison was employed to test significant differences among the levels of the factors investigated. I analyzed survival data with two methods. First, I used the Kaplan-Meier survival analysis (Kaplan and Meier, 1958) to examine whether survival curves of these three species differed significantly at each temperature treatment. If significant difference was found, I further examined the survival curves between either 2 of these species with an alpha (α) value adjusted at 0.017 (= 0.05/3) (Mantel-Cox method). Second, I used chi-square test or Fisher's exact test to analyze whether the 3 months survival rate differed significantly among species at each temperature treatment.

On body mass data, I used a mixed linear model (Norušis, 2004) to evaluate the effects of temperature treatments, exposure time, and interaction on body mass for each species. I performed this analysis in two steps. First, I examined whether body mass changes of lizards kept in H or EH treatments differed from that of lizards kept in C treatment by setting a regression model (Full Model), at which C treatment was treated as the baseline group. The full model was formulated as: $\text{Body mass (g)} = \text{intercept} + \beta_1 \text{ week} + \beta_2 \text{ H treatment} + \beta_3 \text{ EH treatment} + \beta_4 \text{ week} \times \text{H treatment} + \beta_5 \text{ week} \times \text{EH treatment}$; where β_s are estimated coefficients for each predictor. H and EH treatments were coded as dummy variables. Then, I examined whether lizards in H and EH treatments differed significantly in their body mass change. I built another regression model (Reduced Model) as following: $\text{Body mass (g)} = \text{intercept} + \beta_6 \text{ week} + \beta_7 \text{ treatment} + \beta_8 \text{ week} \times \text{treatment}$, where the dummy variable "treatment" refers to both H and EH treatments and β_s are estimated coefficients for each predictor. The Deviance test was used to compare the best of fit for these two models (Singer and Willett, 2003).

When the deviance difference between these two models exceeded statistical level, it meant that lizards in H and EH treatments differed significantly in their body mass change. I used the maximum likelihood (ML) estimation for estimation of the regression coefficients. All statistic tests were conducted with Statistical Package for Social Science (SPSS), version 13.0. All values are shown as mean \pm 1 standard error (SE).

RESULTS

Critical Thermal Maximum (CTMax)

The means of LRR and OS of these three species acclimated at 30 °C fell in ranges between 43.4 - 44.1 °C, and 44.3 - 44.5 °C, respectively. The CTMax was significantly affected by effects of species and acclimation temperature, but not by the interaction effect (species: Wilks' $\lambda = 0.59$, $F_{4,176} = 13.0$, $p < 0.001$; temperature: Wilks' $\lambda = 0.84$, $F_{4,176} = 4.01$, $p = 0.004$; interaction :Wilks' $\lambda = 0.89$, $F_{8,176} = 4.01$, $p = 0.26$, $n = 98$). Both LRR and OS of these species increased significantly with acclimation temperatures (both $p < 0.001$), indicating the highest values at the 30 °C treatment, the middle at the 20 °C treatment, and the lowest at the 10 °C treatment (all $p < 0.01$, Scheffé post hoc test; also see Fig. 2). However, a significant difference among these species was only found in their LRR, but not in their OS. Post hoc analysis detected that the significant LRR difference only occurred between *T. hsuehshanensis* and *T. formosanus* ($p = 0.02$), indicating a 0.5 °C higher LRR in *T. hsuehshanensis* than that in *T. formosanus* after controlling for the temperature effect.

Survival rates and body mass change

These three *Takydromus* species did not exhibit significant differences in their survival curves when they were compared in all temperature treatments (all $p > 0.07$, $n = 16-17$ in each treatment, Kaplan-Meier analysis; Fig. 3). Also, their 3 months survival rates were not significantly different from each other in C treatment ($\sim 94\%$, all $p = 1.0$, Fisher's exact test), H treatment (64.7% - 80.0%, all $p > 0.44$, Fisher's exact test), and EH treatment (35.3% \sim 64.7%, $\chi^2 = 3.30$, $df = 2$, $n = 51$, $p = 0.29$, Pearson chi-square test).

Fig. 4 shows the percent change in body weight (%) of these *Takydromus* lizards, which was calculated as: $100\% \times (\text{weight}_{\text{the } i\text{th week}} - \text{weight}_{\text{the first week}}) / (\text{weight}_{\text{the first week}})$. The body mass of *T. hsuehshanensis* was not affected significantly by time, temperature treatment, or their interactions (all $p > 0.10$, $n = 32$; Table 1; Fig. 4a). On contrary, the body mass of the other two species was affected by the interaction of time and temperature treatment (Table 1). In the case of *T. formosanus*, the body mass did not change significantly over time in C treatment, but it increased significantly in a linear trend in both H and EH treatments (both $p < 0.04$, $n = 38$) (Table 1; Fig. 4b). The rates of their body mass increase did not significantly vary in H and EH treatments (Deviance difference = 0.9, $df = 4$, $p = 0.92$, chi-square test). As for *T. stejnegeri*, their body mass did not change significantly over time in C and H treatments ($p > 0.50$), but it increased significantly in a linear trend in EH treatment ($n = 40$, Table 1; Fig. 4c). Also, lizards in EH treatment had a significantly higher rate in body mass increase than those in H and C treatment (Deviance difference = 11.65, $df = 4$, $p = 0.02$, chi-square test).

DISCUSSION

I did not find a significant CTMax difference among the three *Takydromus* lizards in any of the acclimation temperature treatments. Previous studies have been shown that the behavioral thermoregulation of lizards tends to reduce the importance of physiological adjustment to compensate for low environmental temperature (Hertz, 1981; Hertz and Huey, 1981; Huey and Kingsolver, 1989; Adolph, 1990; Gillis, 1991). Therefore, whether CTMax differentiated among ectothermic vertebrates living at different altitudes might be related to their behavioural thermoregulation. In Table 2, I summarized studies concerning CTMax and behavioral thermoregulation in ectothermic vertebrates. It shows that most reptile species reveal no significant CTMax difference among populations living at different altitudes, except for 2 *Anolis* species (*A. gundlachi* and *A. semilineatus*) (Hertz, 1979; Hertz et al., 1979). Conversely, most amphibian species show a significant CTMax difference among populations, except for *Ambystoma macrodactylum* (Howard and Wallace, 1983). The reason for this difference between amphibian and reptile species might be due to their thermoregulatory behavior. Compared to reptiles, amphibians are poor thermoregulators (Brattstrom, 1963). They exhibit activity temperatures that can be predicted accurately from environmental temperatures and match well the expected altitudinal thermal cline (Navas, 1996). Actually, without thermoregulatory behavior to compensate the temperature difference among an altitudinal or latitudinal cline, amphibians have been shown to exhibit a more prominent geographical variation in their sensitivity to environmental temperatures than that of reptiles (Brattstrom, 1968; Miller and Packard, 1974, 1977; Hoppe, 1978).

The above rationalization was previously presented by Hertz (1981) who proposed

that “species which use thermal regulatory behaviours to compensate for altitudinal changes in the thermal environment exhibit less geographical variation in their sensitivity to high temperatures than does the thermoconformers”. It explains why *A. gundlachi* and *A. semilineatus*, two thermoconformer reptiles, showed a significant CTMax difference among its populations while most other reptiles did not exhibit a CTMax differentiation among their populations at different altitudes.

Besides population levels, I found that a similar relationship between CTMax differentiation and behavioral thermoregulation also occurs among species living at different altitudes (see Table 2). For example, for poorly thermoregulating amphibians, *Eleutherodactylus richmondi* (Heatwole et al., 1965), *Bufo bocourti* (Stuart, 1951) and *Bufo bankorensis* (Chen et al., 2004; Wei and Hou, 2004), CTMaxs were lower than those of their lowland congeners. Also, the CTMax of the mountainous skink (*Sphenomorphus taiwanensis*) in Taiwan was lower than its lowland congener *S. incognitus* (Huang et al., 2006). The *S. taiwanensis* is a common lizard, but a poor behavioral thermoregulator. With its slender body and relatively small legs, it usually hides under natural shelters (rocks or woods) and rarely basks on open surfaces (Huang et al., 2005). Conversely, good thermoregulators, such as the mountainous lizard *T. hsuehshanensis* and the snake *Trimeresurus gracilis* (Huang et al., 2007), did not exhibit lower CTMaxs in comparison with their lowland congeners. Both of these two species were usually observed basking in open areas. Therefore, I conclude that CTMax differences among species living at different altitudes might correlate to their behavioural thermoregulation. If this conclusion is correct, I also predict that mountainous ectothermic vertebrates whose body temperature is apparently influenced by ambient temperature, such as those crepuscular,

nocturnal and fossorial species, might differentiate their CTMax from lowland congeners. More studies are required to strengthen this conclusion.

The *T. hsuehshanensis* and *S. taiwanensis* are the two most abundant lizards in high mountainous areas of Taiwan. An apparently higher CTMax in *T. hsuehshanensis* (44.1 °C) than in *S. taiwanensis* (38.4 °C, Huang et al., 2006) might also explain their basking behaviour and habitat selection. For example, the typical natural habitat of *T. hsuehshanensis* is in the marginal areas of mountainous forests, which are mainly composed of an endemic dwarf bamboo. There, it shuttles between open sunny places and shade to regulate their body temperatures. A higher heat tolerance could protect it from accidentally over heating during thermoregulation. On the other hand, the typical natural habitats of *S. taiwanensis* are restricted to dense forests and its marginal areas. These habitats are relatively cooler and wetter than those of *T. hsuehshanensis*. The *S. taiwanensis* usually remains hidden under rocks or logs and is rarely found basking even on sunny days (Huang et al., 2005). Similar finding regarding the correlation of CTMax to habitat types was also reported on *Anolis* lizards (Hertz, 1979).

In this study, I found that *T. hsuehshanensis* was able to tolerate high temperatures approximating those of lowland areas for short and prolonged periods of time. Furthermore, the heat tolerance of these three *Takydromus* lizards did not differ from each other. Thus, the heat tolerance of *T. hsuehshanensis* is not a crucial factor limiting its current altitudinal distribution. Contrary to this finding, previous studies have been shown that heat tolerance might be an important factor limiting the altitudinal range of mountainous *S. taiwanensis* (Huang et al., 2006). Although altitudinal distribution of *T. hsuehshanensis* could not be explained by its heat tolerance, temperature might still affect

its altitudinal ranges through other aspects of thermal physiology. For example, locomotor performance has been shown to be affected prominently by body temperatures (van Berkum, 1988) and has a crucial impact on animals' survival in the field (Christian and Tracy, 1981; Jayne and Bennett, 1990). Also, thermal requirements of embryonic development may be related to geographic distributions (Lourdais et al., 2004). Further investigations on influences of temperatures on thermal performances and/or fecundity of the *T. hsuehshanensis* are needed to examine the role of temperature on their altitudinal distributions.

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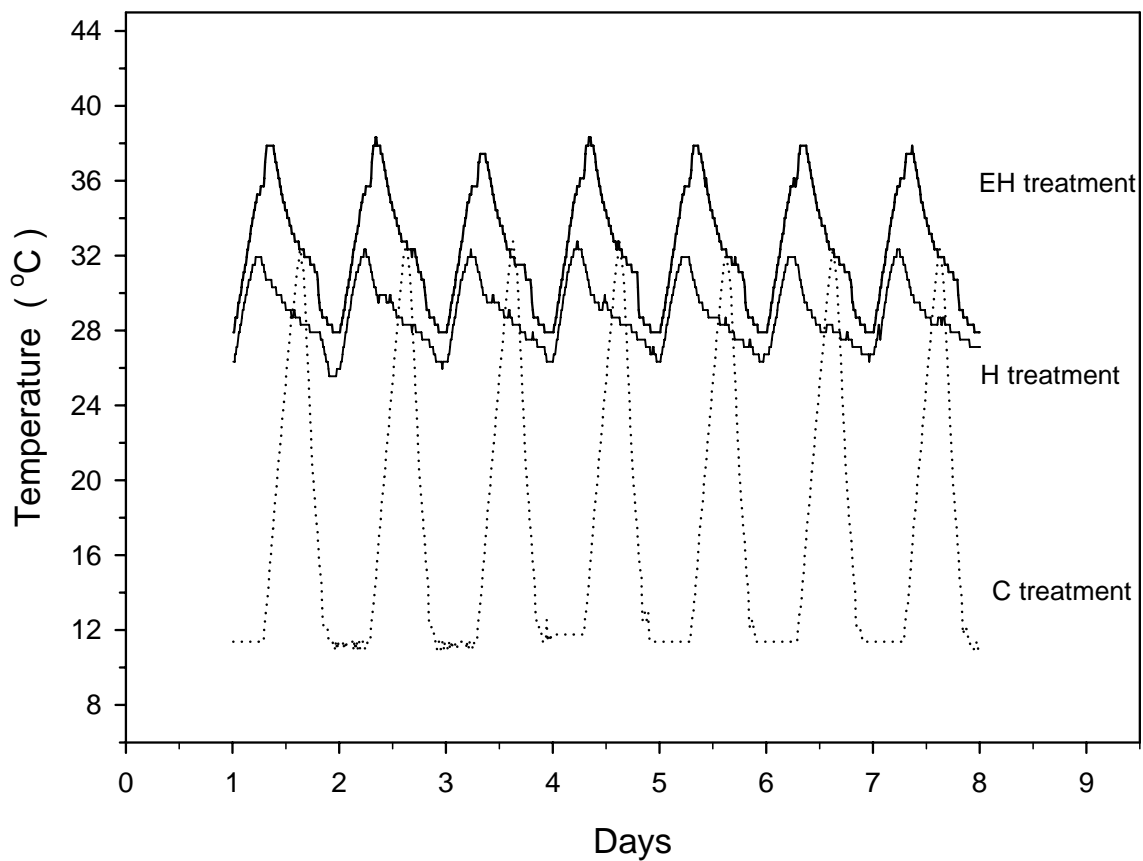


Fig. 1. Temperature recordings in 3 thermal incubators set cool (C), high (H), and extremely high (EH) treatments. C treatment: mean temperature = 17.3 ± 0.09 °C, daily range = 10.8 ± 0.07 °C ~ 32.1 ± 0.05 °C (n = 63 days); H treatment: mean temperature = 28.9 ± 0.04 °C, daily range = 26.5 ± 0.06 °C ~ 32.6 ± 0.06 °C (n = 59 days); EH treatment: mean temperature = 32.8 ± 0.07 °C, daily range = 28.7 ± 0.05 °C ~ 38.0 ± 0.06 °C (n = 79 days). Values are shown in mean \pm 1 SE.

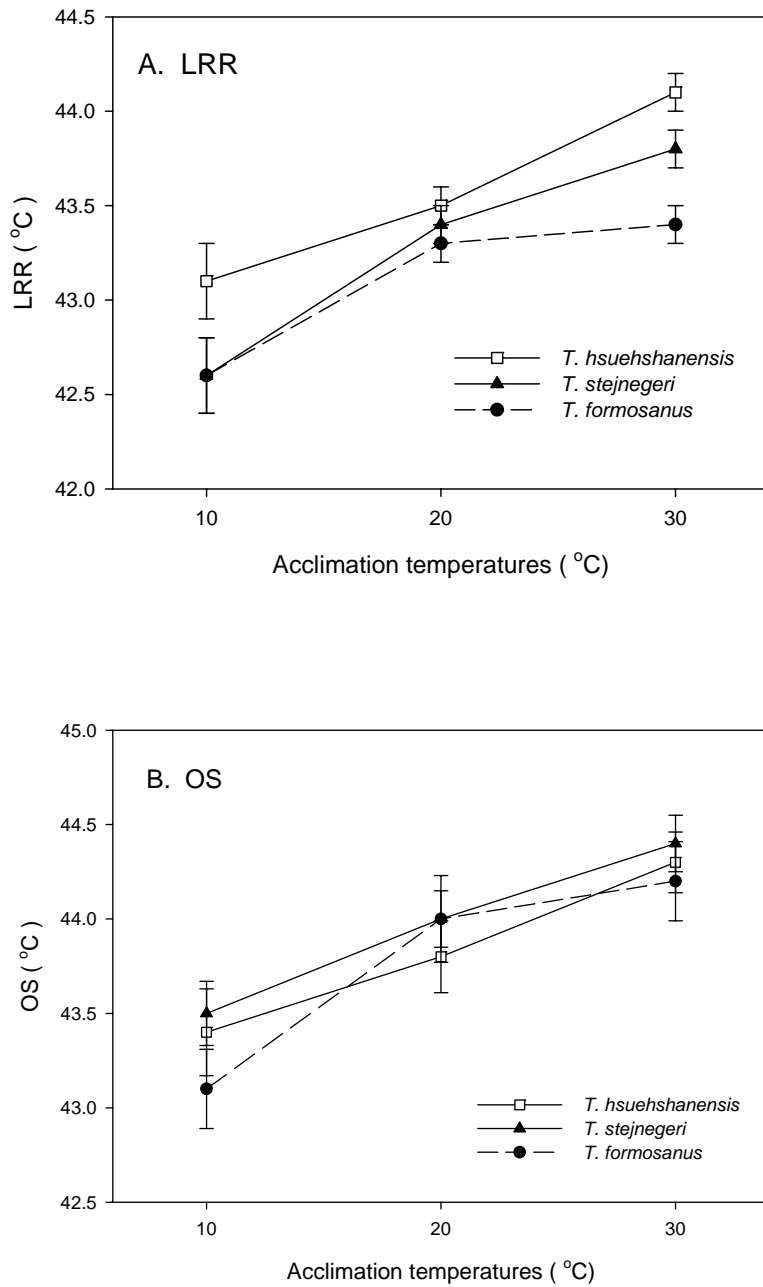


Fig. 2. CTMax (mean \pm 1 SE) of males of three *Takydromus* species after a 2 week acclimation period at 10, 20, and 30 °C. Fig (A), losing righting reflex; Fig (B) onset of spasm. The sample sizes for *T. hsuehshanensis*, *T. formosanus*, and *T. stejnegeri* were 31, 24, and 44, respectively.

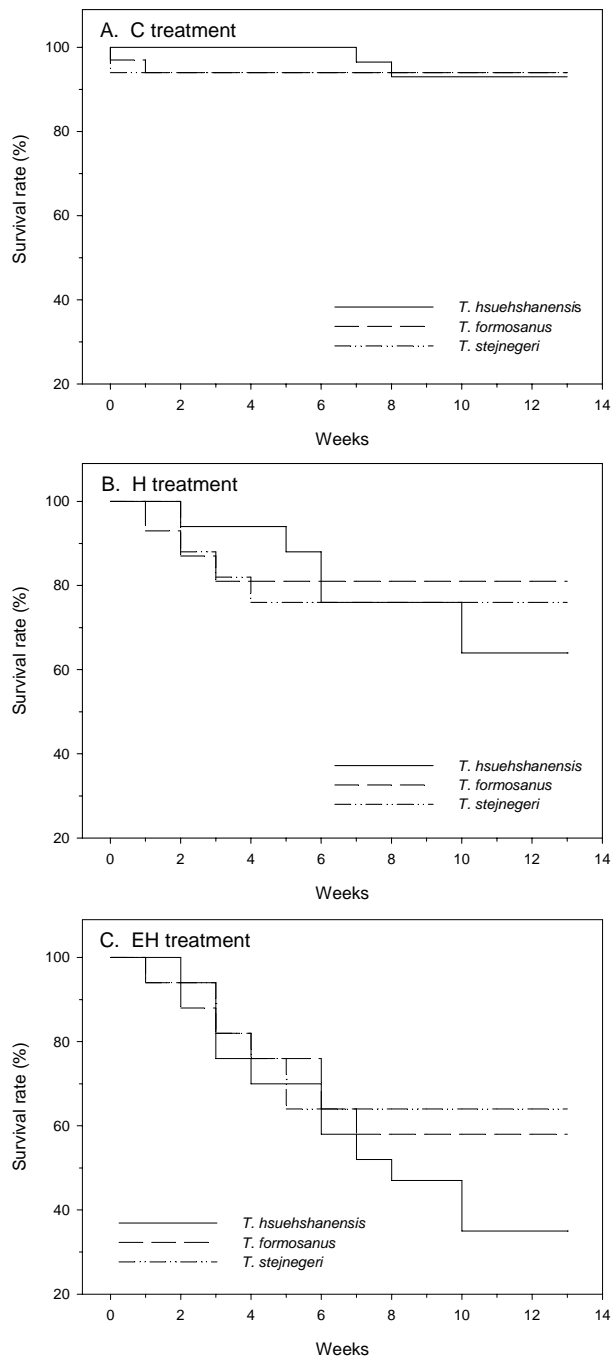


Fig. 3. The survival curves for three *Takydromus* lizards in three temperature treatments.

A: Cool (C) treatment, B: Heat (H) treatment, and C: Extremely heat (EH) treatment.

The sample size for each species in each treatment was 16 ~ 17.

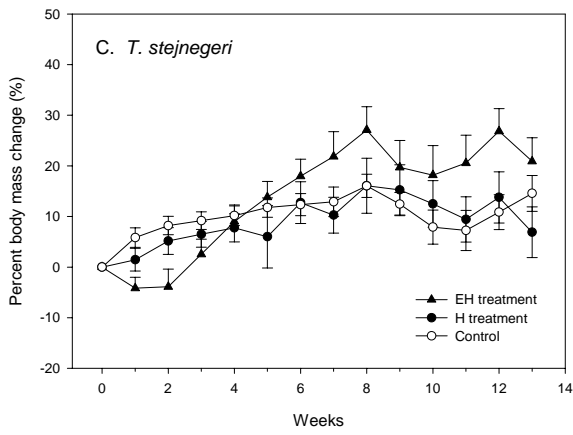
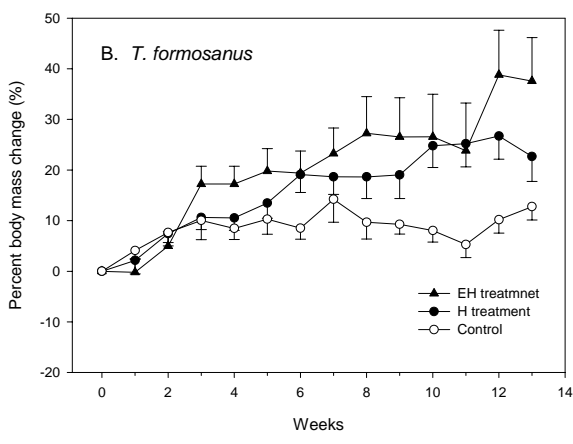
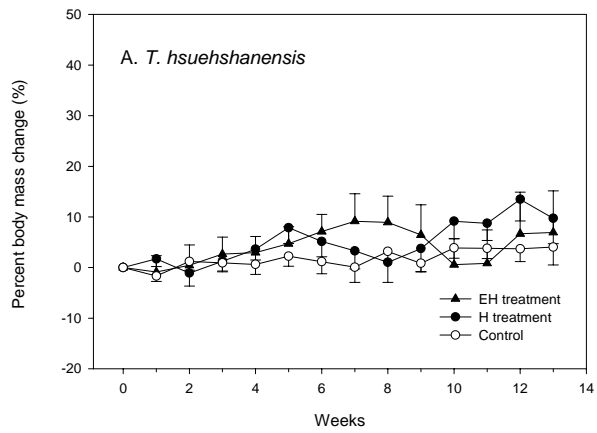


Fig. 4. Percentage of body mass change (mean \pm 1 SE) of three *Takydromus* lizards in 3 different temperature treatments during a 13 week period.

Table 1. Result of mix model regression analysis on body mass for 3 *Takydromus* lizards over 13 weeks. The regression model (Full model): body mass (g) = intercept + β_1 week + β_2 H treatment + β_3 EH treatment + β_4 week \times H treatment + β_5 week \times EH treatment. The cool treatment was treated as the reference group.

Parameters	coefficient	Std. Error	t	p value
<i>T. hsuehshanensis</i>				
intercept	4.91	0.25	18.93	<0.001
treatment EH	-0.19	0.36	-0.54	0.58
treatment H	-0.12	0.35	-0.35	0.72
week	-0.005	0.02	-0.27	0.78
EH treatment \times week	0.04	0.03	1.41	0.16
H treatment \times week	0.02	0.03	0.85	0.40
<i>T. formosanus</i>				
intercept	2.89	0.11	48.24	<0.001
EH treatment	-0.00	0.17	48.15	0.99
H treatment	-0.01	0.16	48.02	0.93
week	0.01	0.01	44.91	0.32
EH treatment \times week	0.05	0.01	45.67	0.006 ^A
H treatment \times week	0.03	0.01	45.82	0.04 ^A
<i>T. stejnegeri</i>				
intercept	3.14	0.14	22.00	<0.001
EH treatment	-0.03	0.20	-0.15	0.88
H treatment	-0.08	0.20	-0.39	0.69
week	-0.01	0.01	1.24	0.22
EH treatment \times week	0.04	0.01	3.34	0.002 ^A
H treatment \times week	0.01	0.01	0.60	0.55 ^B

Note: Different letters, A and B in p value column indicate a significant difference between the coefficients of treatment EH \times time and that of treatment H \times time (a detailed statistical result from Deviance test was reported in the Results section).

Table 2. CTMax difference in reptiles and amphibians living at different altitudes.

Species	Distribution altitudes	CTMax difference	Behavioral thermoregulation	Reference
Amphibians				
<i>Ambystoma tigrinum</i>	lowland, mountain (data not shown)	yes	poor	Delson and Whitford, 1973
<i>Ambystoma macrodactylum</i>	420 m, 1140 m, 2470 m	no	poor	Howard and Wallace, 1983
<i>Bufo</i> toad		yes	poor	Stuart 1951
<i>B. marinus</i>	< 1500 m			
<i>B. bocourti</i>	> 1700 m			
<i>Bufo</i> toad	tadpole and adult	yes	poor	Chen et al., 2004; Wei and Hou, 2004
<i>B. melanostictus</i>	< 500 m			
<i>B. bankorensis</i>	> 1700 m			
<i>Eleutherodactylus portoricensis</i>	15 m, 457 m	yes	poor	Heatwole et al., 1965
<i>Eleutherodactylus</i> frogs		yes	poor	Heatwole et al., 1965
<i>E. richmondi</i>	457 m			
<i>E. portoricensis</i>	15m, 457m			
<i>Pseudacris triseriata</i>	adult: 1542-1631m, 2760-3040 m; tadpole: 1530 m, 2770 m	yes	poor	Hoppe, 1978; Miller and Packard, 1974; 1977
Lizards				
Anolis lizards				
<i>Anolis cristatellus</i>	sea level, 350m	no	good	Huey and Webster, 1976
<i>Anolis gundlachi</i>	270-850 m transect	yes	poor	Hertz et al., 1979
<i>Anolis roquet</i>	20-650 m transact	no	good	Hertz, 1981
<i>Anolis semilineatus</i>	5 m, 535 m, 1190 m	yes	poor	Hertz, 1979
Sphenomorphus lizards				
<i>S. quoyi</i>	8 m, 381 m			
<i>S. tympanum</i> (cool temperate)	381 m, 407 m, 975 m	no	good	Spellerberg, 1972b
<i>S. tympanum</i> (warm temperate)	15 m, 259 m			
<i>S. kosciuskoi</i>	1067 m, 1676 m			
Sphenomorphus lizards				
<i>S. taiwanensis</i>	> 2000 m	yes	poor	Huang et al., 2006
<i>S. incognitus</i>	< 500 m			

^A Losing righting reflex was used as endpoint behaviour criteria for CTMax, and was measured after acclimated at certain temperatures in these studies. (continued)

Table 2. CTMax difference in reptiles and amphibians living at different altitudes

(continued)

Species	Distribution altitudes	CTMax ^A difference	Behavioral thermoregulation	Reference
<i>Takydromus</i> lizards				
<i>T. hsuehshanensis</i>	> 1800 m	no	good	present study
<i>T. formosanus</i>	< 1500 m			
<i>T. stejnegeri</i>	< 1000 m			
<i>Stellio stellio ssp.</i>	170 m, 920 m, 1500 m	no	good	Hertz and Nevo, 1981
<i>Zootoca vivipara</i>	250 m-1450 m transect	no	good	Gvoždík and Castilla, 2001; Hertz et al., 1993
Snakes				
<i>Trimeresurus</i> snakes				
<i>T. gracilis</i>	> 2000 m	no	good	Huang et al., 2007
<i>T. s. stejnegeri</i>	< 1500 m			
<i>T. mucrosquamatus</i>	< 500 m			

^A Losing righting reflex was used as endpoint behaviour criteria for CTMax, and was measured after acclimated at certain temperatures in these studies.

Chapter 3

Cold tolerance and altitudinal distributions of 3 *Takydromus* lizards in Taiwan

Zoological Studies 47 (2008) 438-444.

INTRODUCTION

The geographical distributions of ectotherms may be influenced by a variety of environmental factors, such as temperature, humidity, and oxygen content (Campbell and Solórzano 1992, Krebs 1994, Gaston 2003). Among these factors, temperature is particularly important (Stuart 1951, Graham et al. 1971, Huang et al. 2006) because the body temperature of ectotherms is largely dependent on heat exchange with the physical environment (Pough 1980). Although ectotherms are capable of behaviorally regulating their body temperatures (Brattstrom 1965 1970a 1979, Spellerberg 1972, Huey and Pianka 1977, Hertz and Huey 1981, Huey 1982) to reduce the impacts of ambient temperatures, their physical environments eventually limit the extent of behavioural thermoregulation (Huey 1974, Huey and Stevenson 1979, Wu and Kam 2005). Since body temperatures dramatically affect a variety of physiological functions and behavioral performance (Bennett 1980, Kaufmann and Bennett 1989, Angilletta 2001), I expected that environmental temperatures might affect the survival of ectotherms and consequently their geographic distribution ranges.

As temperature decreases with altitude, high altitudinal ectotherms experience lower temperatures than their low altitudinal congeners (van Damme et al. 1989 1990, Grant and Dunham 1990, Smith and Ballinger 1994, reviewed by Navas 2003). Thus, a cold

environment might foster cold tolerance for closely related species in higher altitudinal areas. Indeed, there is a positive correlation between altitudinal distribution and cold tolerance of some ectothermic vertebrates (lizards: Spellerberg 1972 1973, Huang et al. 2006; amphibians: Brattstrom 1968). Also, some lowland ectotherms suffered death from direct exposure to cold temperatures that approximated high mountain temperatures (amphibians: Wei and Hou 2004; lizards: Heatwole et al. 1969, Gorman and Hillman 1977, Huang et al. 2006).

I measured the cold tolerance of three *Takydromus* lizard species distributed at different altitudes in Taiwan. Traditionally, the critical thermal minimum (CTMin) is used as an important index of the cold tolerance of animals. It is the body temperature at which an animal's locomotion activity becomes disorganized, and the animal presumably cannot escape from conditions that will promptly lead to its death (Cowles and Bogert 1944, Lowe and Vance 1955, Hutchison 1961). Nevertheless, the CTMin can reveal only the short-term tolerance of an animal. I examined prolonged thermal tolerance as well because an animal might be able to tolerate cold temperatures for short periods of time but not for long periods of time (Gorman and Hillman 1977, Huey 1982, Huang et al. 2006). Therefore, in this study I measured both the CTMin and 3-month survival rates to provide information on the cold tolerance over different time scales.

I attempted to determine whether cold tolerance was critical in limiting the distribution of two *Takydromus* species at different high altitudes. In Taiwan, *T. stejnegeri* and *T. formosanus* are only found below 1000m and 1500 m, respectively, whereas *T. hsuehshanensis* occurs only above 1800 m (Lue et al. 1999). I measured and compared the cold tolerances, both CTMin and prolonged cold tolerance (i.e., the

survival rate) under cold treatments of these three species. Since an ectotherm's CTMin may change with acclimation temperatures (Brattstrom and Regal 1965, Brattstrom 1970b, Hutchison and Ferrance 1970), the CTMin was measured after a 2-weeks acclimation at 3 temperatures. Their survival rates were assessed under 4 cold treatments over a 3-month period. I also collected data on winter temperatures in a high altitudinal area to infer the impacts of field temperatures on their altitudinal distributions. I predicted that 1) these three *Takydromus* lizards' cold tolerances would correlate with the upper limit of their altitudinal distributions; and 2) *T. stejnegeri* and *T. formosanus* could not survive cold temperatures similar to those of the high altitudinal area, consequently limiting their current altitudinal distributions.

MATERIALS AND METHODS

Animal collection and maintenance

From May to October 2004 and 2005, I collected *Takydromus hsuehshanensis* (female: mass = 4.1 ± 0.1 g, $n = 33$; male: mass = 4.4 ± 0.1 g, $n = 74$) (mean \pm 1 standard error (SE)) from Nantou County. During the same time, I collected *T. stejnegeri* (female: mass = 2.9 ± 0.1 g, $n = 32$; male: mass = 2.6 ± 0.1 g, $n = 87$) from Taoyuan and Taipei Counties and *T. formosanus* (female: mass = 2.9 ± 0.1 g, $n = 32$; male: mass = 2.6 ± 0.1 g, $n = 60$) from Taipei County. I measured the CTMin values in the lizards' active seasons. All females were excluded to prevent a potential pregnancy effect on CTMin values. I tested the survival rate during the winter in both males and females.

Animals collected before end of August were used for the CTMin measurement. Since the capacity of the long-term cold tolerance was importance for lizards to survive through winter in the field, I used the animals collected after August for the prolonged

survival test in the winter. In the laboratory, animals were individually housed in plastic boxes (length \times width \times height of 20 \times 10 \times 15 cm) containing a layer of soil and dry grass as substrates and a piece of tile as a shelter. Animals were kept in a temperature-controlled room ($\sim 25^{\circ}\text{C}$, with a 12: 12-h light (L): dark (D) photoperiod) before the experiments. They were provided with food (crickets and mealworms dusted with vitamin powder) every 3-4 days and water *ad libitum*.

Critical thermal minimum (CTMin)

I measured the CTMin from June to September 2005. I assigned individuals of each species to three temperature groups of 10, 20, and 30°C ($n = 12-16$ in each group). Each group of the same species contained lizards of approximately the same mean body weight. CTMin was measured after a 2-week acclimation period at the assigned temperature with a 12: 12-hr L: D photoperiod. Lizards were starved for 2 days prior to the CTMin measurements.

The CTMin is defined as the body temperature at which the animal loses its righting reflex (Hutchison 1961). To measure the body temperature, I inserted a thermocouple probe (K type, 0.1 mm in diameter) approximately 1 cm into the lizard's cloaca. The thermocouple probe connected to a data logger (RS-232, Thermolog 302, Center Technology Corp., Taiwan) was secured to the animal's tail with surgical tape. I measured the CTMin in a 0°C walk-in chamber. I put an experimental lizard into a test box (length \times width \times height of 17 \times 10 \times 10 cm). I used a light bulb (60 W) as a heat source to generate a thermal gradient. By gradually moving the lamp further away from the lizard, I decreased the lizard's body temperature by $0.6-0.9^{\circ}\text{C}/\text{min}$. Once the body temperature

fell below 10°C, I checked the lizard's righting reflex every 30 seconds by turning the lizard on its back and stimulating the pelvic region. If the lizard could not right itself within 1 minute, its body temperature was recorded as the CTMin. Immediately after I obtained the CTMin, I placed the lizard in warm water (~25°C). All lizards recovered within a few minutes.

Survival rates under cold treatments

I carried out prolonged survival tests during the winters of 2004 and 2005. Before I recorded the survival rates of these three *Takydromus* species, lizards were kept in a temperature-controlled room at 25°C. For the experiment, I used temperatures of 2, 5, 10, and 15°C. Each treatment group contained lizards of approximately the same mean body weight ($n = 16-17$ for each treatment). Animals were individually housed in plastic boxes (length \times width \times height of 20 \times 10 \times 15 cm) containing dry soil, grass, and a tile shelter. They were put in incubators whose temperatures were decreased from 25°C to their assigned temperature settings at a rate of 1°C/day. Lizards were provided water and food (mealworms dusted with vitamin powder) *ad libitum* during the test, with a 12: 12-h L: D photoperiod. I recorded their survival state weekly.

To compare the experimental temperatures to field temperatures, I measured field temperatures at a high altitudinal plot (Kuanyuan, 2374 m in altitude) from December 2004 to March 2005. This plot was located in the natural range of *T. hsuehshanensis*. Soil temperatures of the surface (5 cm deep) and at a depth of 50 cm were recorded every 30 minutes with Hobo thermal data loggers (model H08-032-08, Onset Computer Corporation, Borne, MA, USA).

Data analysis

I used two-way analysis of variance (ANOVA) to analyze the effects of acclimation temperature and species on CTMin. The CTMin of lizards acclimated to 10°C was used as the lowest CTMin of these three species in the following tests. I compared the lowest CTMin values and their variances with the following hypotheses using the ordered heterogeneity (OH) test (Gaines and Rice 1990, Rice and Gaines 1994). The variance of CTMin was supposed to be smaller in *T. hsuehshanensis* because CTMin was presumably under strong natural selection by the cold weather in the high altitudes; comparably, it was larger in *T. stejnegeri* because CTMin of *T. stejnegeri* was presumably not under strong selection by the warmer climate in the winter. The null hypothesis stated that the lowest CTMin values and their variances were equal among the three species, while the alternative hypothesis stated that the rank of the lowest CTMin and its variance was $T. hsuehshanensis \leq T. formosanus \leq T. stejnegeri$ with at least one inequality strict. Differences in survival curves among these three species at each temperature treatment were examined by a Kaplan-Meier survival analysis (Kaplan and Meier 1958). All tests were conducted with the Statistical Package for Social Science (SPSS), version 13.0.

RESULTS

Critical thermal minimum (CTMin)

The CTMin was significantly affected by species ($F_{2,109} = 32.20, p < 0.001$) and acclimation temperature ($F_{2,109} = 15.07, p < 0.001$), but there were no interaction effects ($F_{4,109} = 1.41, p = 0.23$). For the species effect, at all acclimation temperatures, *T. hsuehshanensis* had a significantly lower CTMin than did *T. stejnegeri* and *T. formosanus* (both $p < 0.001$), but those of *T. stejnegeri* and *T. formosanus* did not significantly differ ($p = 0.76$). For the temperature acclimation effect, the CTMin values of these *Takydromus* lizards were significantly higher at 30°C than at 10 or 20°C, but they did not significantly differ between 10 and 20°C treatments (Fig. 1).

The lowest CTMin and its variance of these lizards had the following rank: *T. hsuehshanensis* (mean \pm 1 standard deviation (SD) = $2.0 \pm 0.7^\circ\text{C}$) \leq *T. formosanus* ($4.5 \pm 1.9^\circ\text{C}$) \leq *T. stejnegeri* ($5.0 \pm 2.1^\circ\text{C}$) (CTMin: ANOVA test, $F_{2,39} = 18.40, p < 0.001$, OH test: $\text{rsPc} > 0.99, p < 0.001$; CTMin variance: Levene's test, $F_{2,37} = 5.03, p = 0.01$; OH test: $\text{rsPc} = 0.88, p < 0.02$).

Survival rates during cold treatments

The survival curves of these three *Takydromus* lizards significantly differed at 2 and 5°C, but not at 10 and 15°C (both $p > 0.50$) (Fig. 2). At 2°C, *T. hsuehshanensis* had a significantly different survival curve from those of the *T. formosanus* and *T. stejnegeri* (both $p < 0.001$), but the survival curves of the latter two species did not significantly differ ($p = 0.90$). At 5°C, survival curves of *T. hsuehshanensis* and *T. formosanus* significantly differed from that of *T. stejnegeri* (both $p < 0.01$), but they did not

significantly differ from each other ($p = 0.89$).

The temperatures recorded at the Kuanyuan plot are shown in figure 3. Most of the time, temperatures of the soil at 50 cm underground were higher than 5°C with the exception of 2 consecutive days. The lowest temperatures were 1.8 and 3.9°C at the surface and 50 cm underground, respectively. It is clear that the 2 and 5°C treatments in the laboratory experiment were much more rigorous than the temperature of 50 cm underground in the field at high altitude.

DISCUSSION

I found a significant acclimation temperature effect on the CTMin values of these three *Takydromus* lizards, which is consistent with many previous studies on ectotherms (Lowe and Vance 1955, Hutchison 1961, Brattstrom 1968, Jacobson and Whitford 1970, Kour and Hutchison 1970). In this study, I took the CTMin at 10°C as the lowest CTMin for further comparison because CTMin values for each species at 10°C did not significantly differ from those of lizards at 20°C (Fig. 1). Because CTMin was correlated with altitudinal distribution in these three lizards and because a comparative low variation of CTMin was found in *T. hsuehshanensis*, I suggested that the cold temperature was a strong selection factor in high altitude. It seemed that the CTMin should be correlated to relevant physiological traits that directly affected survivorship, but it was not itself directly important to the survivorship of these three species.

As predicted, I detected a positive correlation between cold tolerance and the altitudinal distributions of these three species. This finding is also consistent with

previous studies that have showed a lower CTMin in ectothermic tetrapods living in cooler environments, such as higher altitudes (amphibians: Stuart 1951, Brattstrom 1968; reptiles: Spellerberg 1972, Huang et al. 2006) or latitudes (amphibians: Brattstrom 1968; reptiles: Wilson and Echternacht 1987). However, some studies did not detect the same pattern among populations of a single species (Gvoždík and Castilla 2001) or among closely related species of reptiles living at different altitudes (Huang et al. 2007).

Although cold tolerance was highly correlated to the altitudinal distributions of these species, it was not a critical factor limiting the current altitudinal distributions of *T. formosanus* and *T. stejnegeri*. The lowest CTMin values of these two related species were between 4.5 and 5.0°C. At 5°C, 80% of individuals survived at least 6 weeks. Even at 2°C, more than 60% of individuals survived for at least 4 weeks (Fig. 2). Although the surface temperature at the high altitude (2374m in altitude) fell below 2°C in winter, the soil temperature 50 cm underground exceeded 5°C most of the time with only the exception of 2 consecutive days (Fig. 3). Obviously, the low temperatures I observed at the high altitude would not freeze all individuals of these lizards. Therefore, if these two species are capable of moving to high altitudes, at least some individuals might survive the winter. In addition, I found the CTMin values of these two species did not significantly differ from that of another high-mountainous lizard, the Taiwanese skink (with a CTMin of 5.3°C, Huang et al. 2006). Therefore, cold temperatures at high altitude did not cause the deaths of these two species acutely or chronically.

The original concept of critical body temperature (i.e., CTMin and CTMax) is the body temperature at which an animal's locomotor activity becomes disorganized, and

presumably it can no longer escape from conditions that will promptly lead to its death (Cowles and Bogert 1944, Lowe and Vance 1955, Hutchison 1961). This concept may be fine for CTMax, but might not be proper for CTMin. For instance, in our previous study, we found that these three *Takydromus* species would die when they reached the CTMax during the CTMax test if we did not immediately cool them (Huang and Tu 2008). In contrast, in the present study, these three species had high proportions of individuals that could survive at temperatures much lower than their CTMin for quite a long time. Whether CTMin is a proper index of ecological death merits further studies.

Although cold temperatures at high altitudes did not have a lethal effect on *T. formosanus* and *T. stejnegeri*, they could still have a negative effect on their altitudinal distribution. Based on the temperatures measured at a high altitude (3005 m; Endemic Species Research Institute, Taiwan), the monthly lowest air temperatures in the active season (May-October 2005 and 2006) were between 1.9-4.2°C. When comparing these with the CTMin of *T. hsuehshanensis* (2.0°C) and that of the other two species (4.5-5.0°C), it is apparent that the two lowland, but not the high-mountainous, species may frequently encounter immobilizing situations at high altitude. Even if *T. formosanus* and *T. stejnegeri* are not immobilized, environmental temperatures may still affect their distributions by altering behavioral performances. In fact, it was reported that tropical lowland frogs lost their normal locomotor capacity at low temperatures normally occurring in high-mountainous areas (Navas 1996 2003). The locomotor performance of ectotherms is prominently affected by body temperature (Bennett 1980, van Berkum 1988) and it crucially impacts a reptile's survival in the field (Christian and Tracy 1981, Jayne and Bennett 1990). I assumed that if the two lowland species exhibit relatively

poor performances at cold temperatures, they may have decreased abilities to compete for resources with the native high-mountainous species, and/or suffer higher mortalities from predation. Further investigations on the thermal performances of these three species are needed to examine the role of temperature on their altitudinal distributions.

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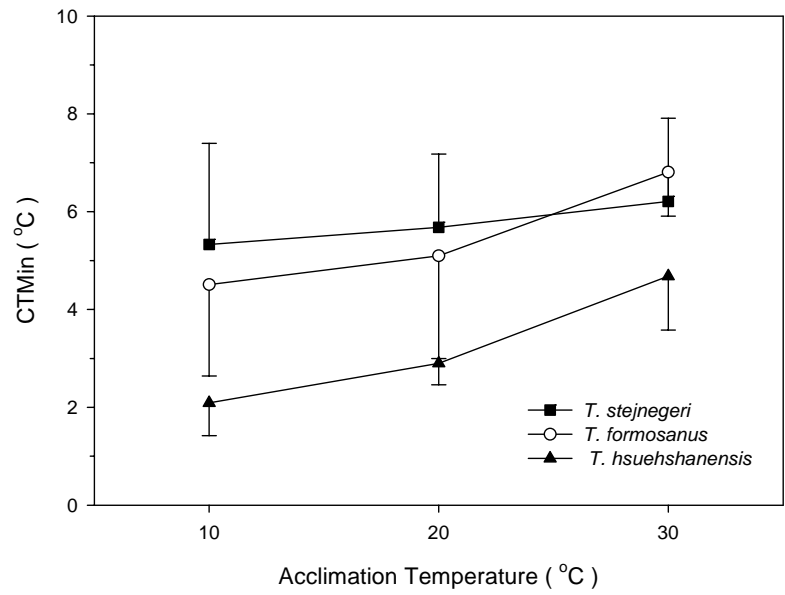


Fig. 1. Critical thermal minimum (mean \pm 1 standard deviation) of three *Takydromus* lizards at 3 acclimation temperatures. Sample sizes for *T. stejnegeri*, *T. formosanus*, and *T. hsuehshanensis* were 16, 12, and 12, respectively.

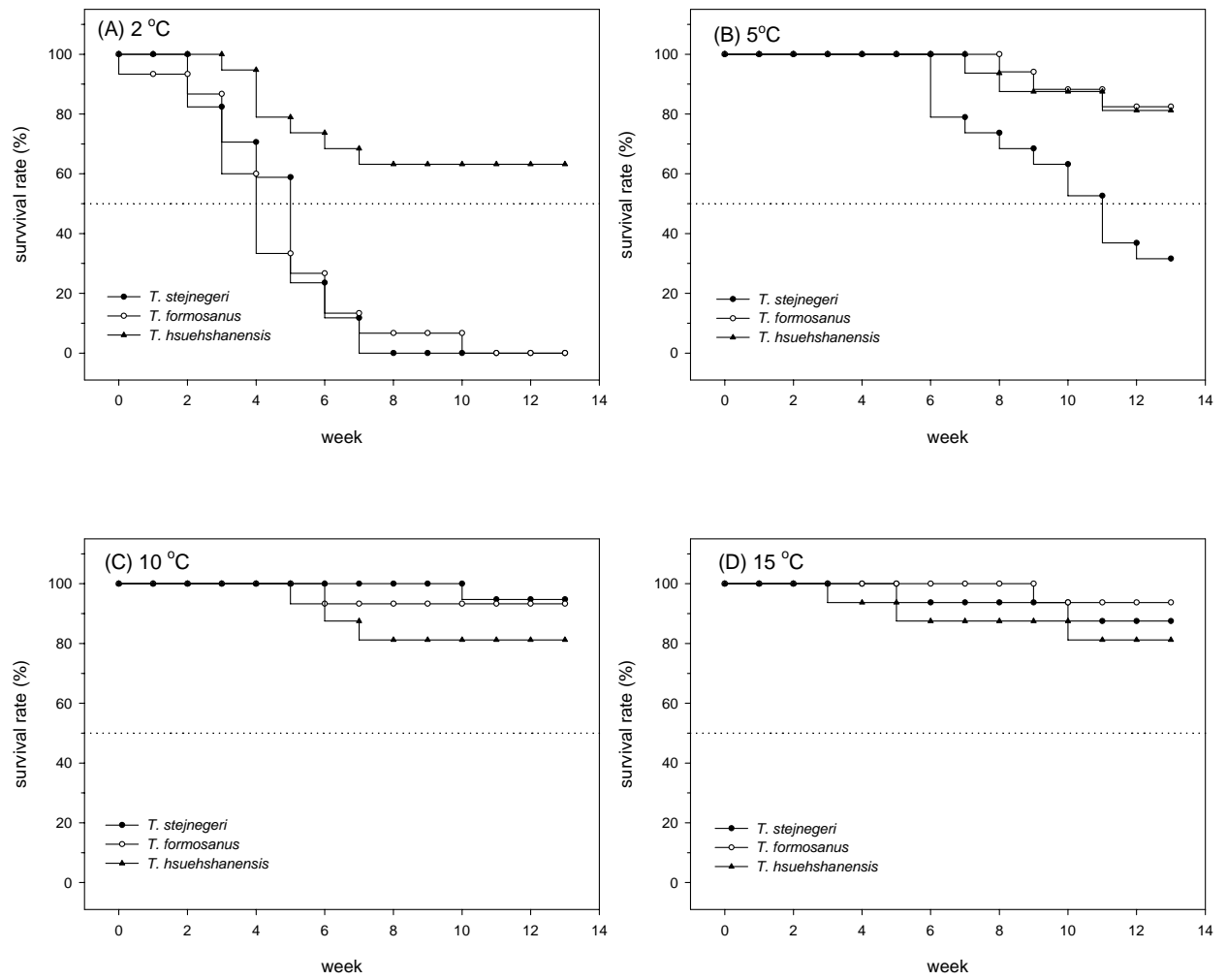


Fig. 2. Survival curves for *Takydromus stejnegeri* (■), *T. formosanus* (○), and *T. hsuehshanensis* (▲) at 4 different temperature treatments. The sample size for each species in each treatment was 16 or 17.

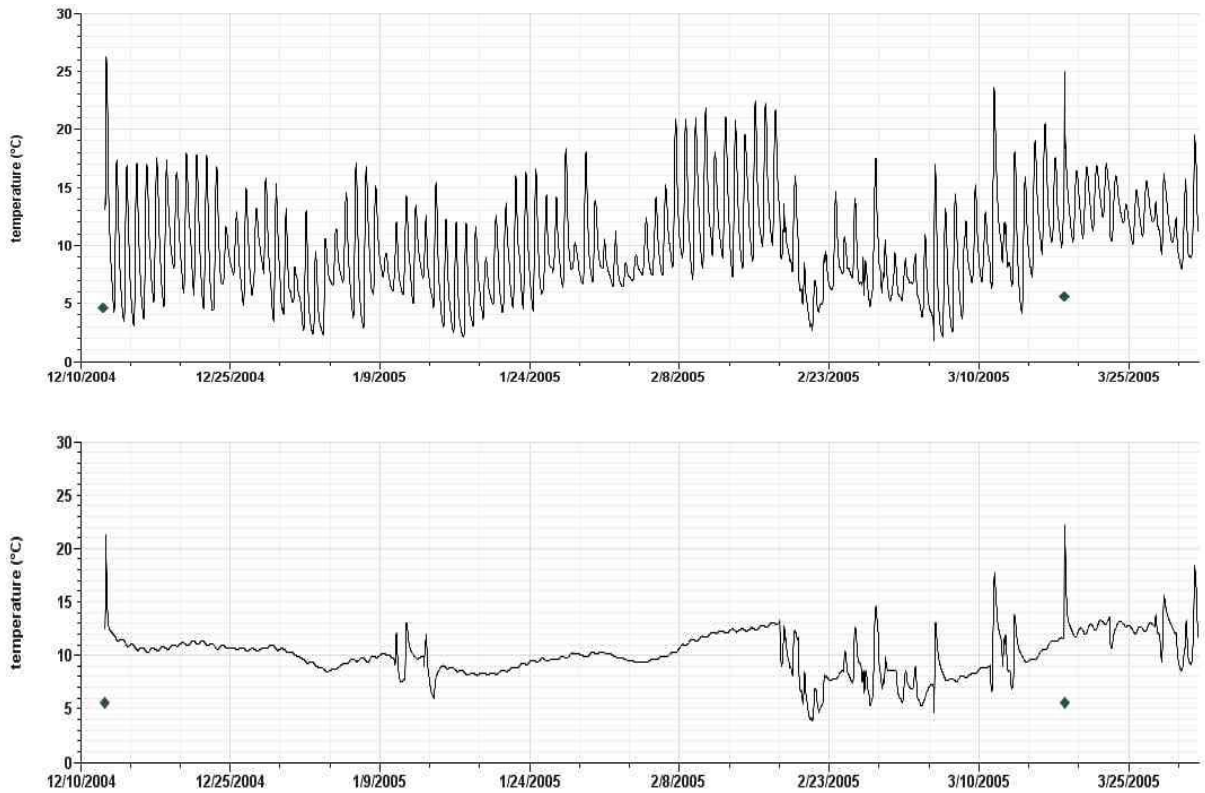


Fig. 3. Temperature recordings at Kuanyuan (2374 m in altitude) from December 2004 to March 2005. Surface temperature (A) and 50 cm underground (B). Diamonds indicate when the laptop was connected to the temperature data logger in order to launch the recordings at the beginning and to read out the data during the measurement period, respectively.

Chapter 4

Locomotor performance and altitudinal distribution of a mountainous lizard,

Takydromus hsuehshanensis, in Taiwan

This chapter has been accepted by 'Zoological Studies'

INTRODUCTION

An species' geographic range is affected by environmental factors (such as temperature, humidity, and oxygen content) or biotic factors (ie. predator. competition, parasitism, and etc)(Krebs 1994). Among the environmental factors, temperature is particularly important to ectotherms' geographic range (Graham et al. 1971, Greer 1980; Huang et al. 2006) because of its impact on the body temperatures of ectotherms (Brattstrom 1965). Body temperature often greatly influences physiological functions and behavioral performance of ectotherms (Bennett 1980, Kaufmann and Bennett 1989).

Temperature changes dramatically with altitude (McCullough and Portor 1971). Although reptiles are capable of adjusting their body temperature (T_b) behaviorally (Spellerberg 1972, Huey and Pianka 1977, Avery 1982, Hertz and Huey 1981, Huey 1982), they may not fully compensate thermal differences among different thermal environments due to associated costs (Huey 1974). Some mountainous reptiles thus have a field T_b lower than their lowland counterparts (Hertz et al. 1983, van Berkum 1986, van Damme et al. 1989). To compensate for the cold temperature effect on their performance, some mountainous ectotherms have physiological adjustments, such as being more tolerant to cold (Heatwole et al. 1969, Huang et al. 2006, Huang and Tu 2008a) or performing better at lower temperatures (Hillyard 1981, Beuchat et al. 1984, Navas 1996) when compared with their lowland counterparts.

The ability of an animal to perform well in specific ecological activities, such as escaping predators or capturing prey, affects its survival, reproduction, and growth (Arnold 1983, Pough

1989, Wainwright 1994). Locomotor performance is a whole-animal measure that is intimately linked to fitness. It affects individuals' fitness in critical activities, such as capturing prey (Jayne and Bennett 1990, Husak et al. 2006) and escaping predators (Christian and Tracy 1981, Husak et al. 2006). Locomotor performance is therefore a relevant ecological measure of physiological performance in ectotherms.

Since locomotor performance of ectotherms is dramatically affected by temperature (Bennett 1980), I proposed that high altitude ectotherms restricted to mountainous areas would have different temperature dependent locomotor capacity to cope with the cold temperature. On the other hand, this different locomotor capacity may hinder these ectotherms from dispersing to lower altitudes. In this study, I seek to find out whether the locomotor capacity of an endemic grasslizard, *Takydromus hsuehshanensis*, is related to its altitudinal distributions. *T. hsuehshanensis* is only distributed at altitudes above 1800m in Taiwan (Lue et al. 1999). We have found that its heat tolerance is not a crucial factor affecting its current distribution (Huang and Tu 2008b). I measured its thermal sensitivity of locomotor performance to see if it is an important factor affecting its altitudinal distributions. I used its closely related species, *T. formosanus* (recently revised as *T. viridipunctatus*, Lue and Lin, 2008), which lives at altitudes lower than 1500m as a reference (Lue et al. 1999). Several important descriptive statistics such as the “optimal” T_b (T_{opt} , herein defined as the best-performance T_b ; see Fig. 1) and the “thermal performance breadth” (B_{80} and B_{80} , herein defined as the range of T_b over which an animal performs well; see Fig. 1) are used to examine their thermal sensitivities (Huey and Stevenson 1979, van Berkum 1986). In addition, to evaluate whether locomotor performance can be adjusted to short term temperature exposures, I also examined their locomotor performance after two weeks of acclimation at two different temperatures (10 and 30°C).

I proposed that locomotor performance of *T. hsuehshanensis* would be impeded at higher temperatures approximating the summer temperatures occurring in lowland areas. When

compared at higher temperatures, *T. hsuehshanensis* was predicted to run slower than *T. formosanus*. As for the thermal sensitivity, because *T. hsuehshanensis* living at a habitat with a more variable and a lower mean temperature, I predicted that *T. hsuehshanensis* would have a lower T_{opt} or broader performance breadth relative to *T. formosanus*. In addition, I predicted that warm acclimated individuals would run faster than cold acclimated individuals when compared at a high T_b , but would run slower than cold acclimated individuals when compared at a low T_b .

MATERIALS AND METHODS

Animal collection

From May to September 2005 and 2007, I collected *T. hsuehshanensis* (mean \pm 1SD)(weight = 4.75 ± 1.1 g; snout-vent length, SVL = 62.6 ± 4.1 mm; n =47) from Mt. Hehuan (2400 ~ 3200 m in altitude, Nantou County). During the same time, I collected *T. formosanus* (mean \pm 1SD)(weight = 2.5 ± 0.4 g, SVL = 50.8 ± 3.1 mm, n = 45) from Taipei County at an altitude lower than 600 m. To prevent a potential pregnancy effect, I only used male lizards in this study. I housed the animals individually in a plastic container (length \times width \times height = 20 cm \times 10 cm \times 15 cm) with a layer of soil and dry grass as substrates; a piece of tile served as a shelter in a constant temperature room (~ 25 °C, Light : Dark = 12hr: 12hr) before the animals were acclimated to temperatures used in our study. Food (crickets and mealworms dusted with vitamin powder) was provided about every 3 days and water was provided *ad lib*. Body mass was monitored before and during the experiment as a health indicator. Lizards that lost over 20 % of their original body mass were excluded (n = 3).

Acclimation temperature treatments

Individuals from each species were assigned randomly into two temperature groups (10

and 30°C). Each group of the same species contained lizards of approximately the same mean body mass. Before taking measurements, they were put into a thermal incubator set at 10 or 30°C, with a photoperiod of L : D = 12hr: 12hr for two weeks. They were provided with food (crickets and mealworms dusted with vitamin powder) every 3 days and water *ad lib*. They were starved for two days before taking measurements to prevent the effect of digestion on locomotor performance

Experimental protocol

I measured the sprint speeds of these lizards at nine different temperatures ranging from 12 ~ 40°C. I assigned 40°C as the highest temperature because it was 3 ~ 4°C lower than their critical thermal maximum (Huang and Tu 2008b). The nine temperature measurements were set in a fixed sequence as follows: 20, 25, 15, 30, 32, 12, 35, 37.5, 20, and 40°C. The order of the first eight settings was randomly chosen, but the order of the last two settings was purposely set. I assigned the 40°C setting last because it might cause a harmful effect on lizards; I used the 20°C setting a second time to verify the health condition of the lizards. If a lizard could not maintain a maximum speed higher than 80% of the first 20°C setting, I would exclude the data collected from that individual; however, I did not need to exclude any for this reason in. Before taking measurements, I placed the test lizards for at least two hours in a temperature controlled walk-in chamber set to the test temperature. Each lizard then ran two trials at these temperature settings except for those at 40°C, which ran only one trial. At 40°C during the preliminary test, I observed some lizards (5 out of 20 tested lizards) showing abnormal behaviors, such as flaccid legs or lolling tongues when they were kept at that setting for more than four hours. Thus, I only took one trial at the 40°C setting. Each trial included three runs, and the tested individuals were given a break for about 4 hours between the trials. Each set of temperature measurements were performed with two days between them. The animals were maintained in their original acclimation temperature settings between each

experimental test.

The sprint speeds were measured using a treadmill (length \times width \times height = 150 cm \times 20 cm \times 25 cm). The treadmill had 16 pairs of infrared timing photocells installed on both of its side walls, with each photocell separated by a distance of 10 cm. I used a soft plastic stick to chase the lizards down the treadmill. The time it took the lizards to run each 10 cm distance was recorded. I calculated the 20 cm sprint speeds by dividing 20 cm by the total time it took the lizard to run every two adjacent 10 cm distances. I then selected the fastest speed among the six runs at each temperature for further analysis. The frequency of maximum speed that occurred in the first and second runs did not significantly differ in these tested individuals ($p=0.65$, chi-square test).

Examination of performance curve

I refer to the performance curve as the relationship between T_b and relative sprint speed (van Berkum 1986, Huey and Kingsolver 1989). For each individual, I transformed its absolute maximum sprint speed into relative sprint speed (%) by dividing it by the fastest sprint speed of this individual measured over all experimental temperatures. I drew a plot of the relative sprint speeds versus temperatures for each lizard by connecting the relative sprint speed at nine test temperatures (as shown in Fig. 1). Following van Berkum (1985), once a value of the sprint speed was lower its two adjacent sprint speeds (i.e. the point 'b' in Fig.1), this value in question was considered too low and was excluded. I then adjusted the questionable data point to an adjusted value by linearly interpolating it from its adjacent pairs of periods (see Fig. 1). When a lizard had more than one sprint speed value in question, I excluded the data of that lizard ($n=4$).

From the performance of each lizard, I extracted the lower and higher bounds of T_b at which a lizard can run at 80% of its fastest speed (i.e. L_{80} and H_{80} , respectively) and at which

the lizard can run at 95% of its fastest speed (i.e. L_{95} and H_{95} , respectively) (see text of Fig. 1). The optimal T_b (i.e. the T_{opt}), defined as the T_b at which a lizard can run its fastest speed, was calculated as the midpoint of the L_{95} and H_{95} . The 80% and 95% performance breadths (i.e. B_{80} and B_{95} , respectively) were calculated by subtracting the H_{80} to L_{80} and H_{95} to L_{95} , respectively.

Data analysis:

To test the effect of species, temperature, and their interactions on the thermal sensitivity, 7 measures (i.e. the L_{80} , L_{95} , H_{80} , H_{95} , B_{80} , B_{95} and T_{opt}) were subjected to multivariate analysis of variance (MANOVA). I analyzed the effect of species, acclimation treatment, and their interaction on the absolute sprint speed, using a two-way repeated MANOVA with absolute sprint speeds measured at 9 body temperatures as the dependent variables. I chose the model using autoregressive heterogeneous structure as the covariance structure after comparing the statistic fit values (AIC and -2LRR) with that of models using other covariate structures (unstructured, compound symmetry, autoregressive). Mixed proc and lsmean/slice of SAS statistics software (version 9.1.3) was used to perform this statistic and to compare the significant difference among groups when the significance level was reached ($\alpha=0.05$). All statistical analysis was performed with SAS software (version 9.1.3).

RESULTS

I did not detect significant effects of species (MANOVA, Wilks' $\Lambda_{5,84} = 0.98$, $p = 0.73$), acclimation temperature (MANOVA, Wilks' $\Lambda_{5,85} = 0.88$, $p = 0.06$), or interaction (MANOVA, Wilks' $\Lambda_{4,85} = 0.73$, $p = 0.60$) on thermal sensitivity of locomotor performance (i.e. L_{80} , H_{80} , L_{95} , H_{95} , B_{80} , B_{95} , and T_{opt}). Table 1 shows these parameters for the locomotor performance of these two species. These two species had a mean L_{80} and a mean H_{80} falling in a range of 30.4 ~ 31.3°C and 37.8 ~ 38.9°C, respectively, depending on which acclimation temperature they

were treated. Their B_{80} ranged from 6.4 ~ 8.2°C. Their mean L_{95} and mean H_{95} were in a range of 33.0 ~ 34.0°C and 35.6 ~ 36.6°C, respectively. The B_{95} ranged from 2.5 ~ 3.0°C, and their mean T_{opt} fell in the range of 34.3 ~ 35.3°C.

Figure 2 indicates the absolute sprint speeds of these two species measured at different T_b . *T. hsuehshanensis* ran significantly slower than did *T. formosanus* ($F_{1,88} = 21.6$, $p < 0.0001$), but there was no significant effect of acclimation temperature treatments ($F_{1,88} = 0.28$, $p = 0.59$) or the interaction ($F_{1,88} = 1.23$, $p = 0.26$) on the absolute sprint speed (two-way repeated MANOVA). Post hoc analysis revealed that *T. formosanus* exhibited a significantly higher sprint speed than that of *T. hsuehshanensis* at T_b above 20°C, but when measured at 12 or 15°C, their sprint speeds were not significantly different from each other.

DISCUSSION

The results falsified our prediction that *T. hsuehshanensis* would have an impeded locomotor performance in high temperature ranges approximating summer temperatures of lowland areas. Its mean T_{opt} fell between the range of 34.3 ~ 35.3°C, which is higher than the regular summer temperatures of lowland areas. According to recordings from the Central Weather Bureau, Taiwan (1971–2000), the monthly mean maximum summer temperature measured in three lowland cities in northern (Taipei), middle (Taichung), and southern (Kaohsiung) Taiwan fell in the range of 31.5 ~ 33.0°C. Clearly, lowland summer temperatures did not exceed the T_{opt} of *T. hsuehshanensis* and thus would not be too high to reduce *T. hsuehshanensis*' locomotor performance. Accordingly, *T. hsuehshanensis*' locomotor performance is not a crucial biotic constraint to affect its altitudinal distribution. Because *T. hsuehshanensis* ran slower than *T. formosanus*, it would probably be at a disadvantage for *T. hsuehshanensis* when it lives sympatrically with *T. formosanus*. Further experiments concerning inter-species competition will be helpful to clarify our interpretation.

I also found these two *Takydromus* lizards did not significantly differ in their thermal sensitivity of locomotor performance. Hertz et al. (1983) have labeled “static” and “labile” views to elaborate the thermal sensitivity of reptilian thermal physiology. The “static” view, traceable to Bogert (1949), postulates that thermal physiology is evolutionarily conservative and resistant to directional selection (i.e. different thermal environments). Results of our study supported the “static” view. Similar findings were reported in other species living at different altitudinal gradients (interspecies comparison: *Agamid* lizards, Hertz et al. 1983; intraspecies comparison: *Sceloporus undulatus*, Crowley 1985; *Podarcis tiliguerta*, van Damme et al. 1989). On the other hand, the “labile” view argues that species or populations do respond to divergent thermal environments by adaptations in thermal physiology. The results regarding the locomotor performance coming from *Anolis* lizards living at different altitude gradients supported the “labile” view (van Berkum 1986).

A possible explanation for the differences in above studies may be related to the effectiveness of thermal regulation behaviors in these ectotherms. Hertz (1981) has proposed that “species which use thermal regulatory behaviors to compensate for altitudinal changes in thermal environment exhibit less geographical variation in their sensitivity to high temperature than do the thermoconformers.” Huey et al (2003) also postulated that thermoregulatory behaviors likely inhibit selection for evolutionary shifts in a lizard (*Anolis cristatellus*). This is true for the similar heat thermal tolerances (the critical thermal maximum) of three *Takydromus* lizards that are distributed in different altitudinal ranges (Huang and Tu, 2008b). I suspect that the similar sprint speeds of these two species could be explained by their effective thermoregulatory behaviors. In Table 2, I collected data regarding locomotor performance of ectotherms that lived in different altitude gradients. I examined whether the difference in thermal sensitivities of locomotor performance among closely related species or populations were related to thermoregulatory behaviors. I defined the behavioral thermoregulators into two

categories, the ‘poor’ or ‘good’. Amphibians were generally considered as poor thermoregulators due to the conflicts of the requirements for respiration and hydroregulation with those for thermoregulation (Hutchison and Dupre 1992). Thus, I classified all amphibians as ‘poor’ behavioral thermoregulators. In respect to reptiles, I defined species that did not have basking behavior as “poor” thermoregulators. I found that effective behavioral thermoregulators, such as some lizards (Crowley 1985, van Damme et al. 1989) followed the “static” view of ectotherms’ thermal physiology. In contrast, those ineffective behavioral thermoregulators, such as some frogs (Navas 1996) and some anoles (van Berkum 1986) followed the “labile view”. Obviously, thermal sensitivity of locomotor performance was closely associated with the effectiveness of behavioral thermoregulation in these ectotherms.

I did not detect significant temperature acclimation effect on the sprint speed of these two *Takydromus* lizards, indicating that their locomotor performance would not be affected by its previous short- term thermal history. These results are consistent with previous studies (anoles: van Berkum 1985; box turtles: Adams 1989; salamanders: Else and Bennett 1987; anurans: Putnam and Bennett 1981, Renaud and Stevens 1983, Whitehead et al. 1989, Knowles and Weigl 1990, Wilson and Franklin 2000). On the contrary, some ectotherms were able to improve locomotor performance based on the temperatures to which they are exposed (fish: Beddow et al. 1995, Johnson and Bennett 1995, Tample and Johnston, 1998; anurans: Wilson and Franklin 1999, toads: Londos and Brooks 1988; salamanders: Feder 1986, Marvin 2003).

In summary, I found that *T. hsuehshanensis* did not have an impeded locomotor performance at high temperatures similar to summer temperatures in lowland areas. Thus, the sprint speed of *T. hsuehshanensis* may not be a crucial factor limiting its current altitudinal distribution. So far, I have demonstrated that neither heat tolerance nor locomotor performance of *T. hsuehshanensis* were critical factors limiting its current altitudinal distribution. Nevertheless, environmental temperature may still have an important role on its altitudinal

distribution through other aspects of thermal physiology. For example, there is a profound thermal effect on embryo development (Packard et al. 1977, Packard and Packard 1988, Chen 2008) and hatchling phenotypes (Booth 2006), which may consequently play important roles in the geographic distributions in reptiles (Parker and Andrews 2007). Further investigations on juvenile survivorship and fecundity are needed to examine the role of temperature on the altitudinal distributions of *T. hsuehshanensis*. Other than temperature effect, biotic factors such as predator, competition, parasitism, may all interact to affect the distribution of animals. By examining each factor step by step, I may eventually clarify the limiting factors of its distribution.

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Table 1. The thermal sensitivity of sprint speed in *T. hsuehshanensis* and *T. formosanus* at 2 acclimation temperatures. L_{95} and L_{80} are the lowest T_b at which lizards are able to run 95% and 80% of maximum sprint speed, respectively; H_{95} and H_{80} are the highest T_b at which lizards are able to run 95% and 80% of maximum sprint speed, respectively; B_{95} and B_{80} are the T_b width at which a lizard is able to run at least 95% and 80% of maximum sprint speed, respectively. Values are means \pm 1 standard error.

species	acclimation temperature	L_{95} ($^{\circ}\text{C}$)	H_{95} ($^{\circ}\text{C}$)	B_{95} ($^{\circ}\text{C}$)	L_{80} ($^{\circ}\text{C}$)	H_{80} ($^{\circ}\text{C}$)	B_{80} ($^{\circ}\text{C}$)	T_{opt} ($^{\circ}\text{C}$)	N
<i>T. hsuehshanensis</i>	10 $^{\circ}\text{C}$	33.0 \pm 0.5	36.4 \pm 0.5	3.0 \pm 0.3	30.7 \pm 0.5	38.9 \pm 0.4	8.2 \pm 0.6	35.0 \pm 0.4	20
	30 $^{\circ}\text{C}$	33.7 \pm 0.4	36.3 \pm 0.4	2.5 \pm 0.3	31.3 \pm 0.4	37.8 \pm 0.3	6.4 \pm 0.5	35.0 \pm 0.4	27
<i>T. formosanus</i>	10 $^{\circ}\text{C}$	33.0 \pm 0.5	35.6 \pm 0.5	2.6 \pm 0.3	30.4 \pm 0.5	38.3 \pm 0.4	7.8 \pm 0.6	34.3 \pm 0.4	21
	30 $^{\circ}\text{C}$	34.0 \pm 0.4	36.6 \pm 0.6	2.5 \pm 0.3	31.5 \pm 0.5	38.2 \pm 0.3	8.2 \pm 0.6	35.2 \pm 0.4	24

Table 2. The thermal sensitivity of locomotor performance and behavioral thermoregulation in some amphibians and reptiles living at different altitudes

species	altitudes	thermal sensitivity parameters		behavioral thermoregulation	reference
		T _{opt} difference	B ₈₀ difference		
Inter-species comparison					
Amphibians					
<i>Atelopus</i>					
<i>A. sp. nov</i>	3500m	--	Yes	Poor	
<i>A. varius</i>	350m			Poor	
<i>Colostethus</i>					
<i>C. subpunctatus</i>	3500m	--	Yes	Poor	
<i>C. flotator</i>	90m			Poor	
<i>Eleutherodactylus</i>					
<i>E. bogotensis</i>	3500m	--	Yes	Poor	Navas 1996
<i>E. diastema</i>	90m			Poor	
<i>Hyla</i>					
<i>H. labialis</i>	2900m	--	Yes	Poor	
<i>H. microcephala</i>	90m			Poor	
<i>H. ebraccata</i>	90m			Poor	
Lizards					
<i>Anolis</i> ¹					
<i>A. tropidolepis</i>	1400-2600m			Poor	
<i>A. limifrons</i>	lowland			Poor	
<i>A. humilis</i>	0-1500m	Yes	Yes	Poor	van Berkum 1986
<i>A. lemurinus</i>	lowland			Poor	
<i>A. intermedius</i>	1200-1800m			Good	
<i>A. lionotus</i>	0-1300m			Good	
<i>A. cupreus</i>	low-mid altitudes			Good	
Inter-population comparison					
<i>Podarcis tiliguerta</i>	0-70m, 1450m	No	No	Good	van Damme et al. 1989
<i>Sceloporus undulatus</i>	1750m, 2350-2400m	No ^a	No ^a	Good ^{b,c}	^a Crowley 1985 ^b Kennedy 1958 ^c Behler 1979

Note: ¹The differences of thermal sensitivities among these 7 *Anolis* species were compared together in the original paper. Therefore, I am not able to show the difference of thermal sensitivities among “Good” thermoregulators, or “Poor” thermoregulators, separately.

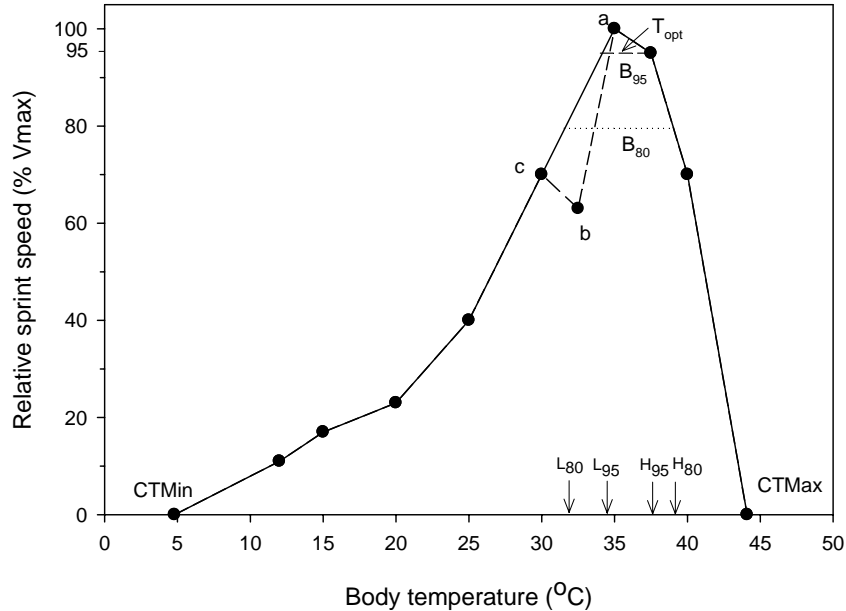


Fig. 1. Schematic diagram illustrating the analysis of sprint speed data and the variables used to describe the thermal sensitivity of the sprint speed. For each lizard, the fastest sprint speed measured at each body temperature (T_b) was transformed to relative speed (see text in methods and materials part). The solid circles indicate the relative sprint speed of a *T. hsuehshanensis* (body weight = 4.45 g, SVL = 63.8 mm). The relative sprint speed is set to zero at mean CTMin and CTMax reported by Huang and Tu (2008b). The point ‘a’ indicates the fastest speed (V_{max} , 100%). Since point ‘b’ is lower than its adjacent points, ‘a’ and ‘c’, it is considered too low and is excluded (van Berkum 1985). As proposed by van Berkum (1986), I drew a line connecting the points ‘a’ and ‘c’ for further analysis. L_{95} and L_{80} are the lowest T_b at which a lizard is able to run 95% and 80% of maximum sprint speed, respectively; H_{95} and H_{80} are the highest T_b at which lizards are able to run 95% and 80% of maximum sprint speed, respectively; B_{95} and B_{80} are the T_b width at which a lizard is able to run at least 95% and 80% of maximum sprint speed, respectively. The T_{opt} is the midpoint of L_{95} and H_{95} , which is defined as the T_b at which a lizard can run the fastest. The dotted lines are performance breadths (Huey and Stevenson 1979), defined as the range of T_b over which a lizard could run 95% (B_{95}) and 80% (B_{80}) of its fastest speed.

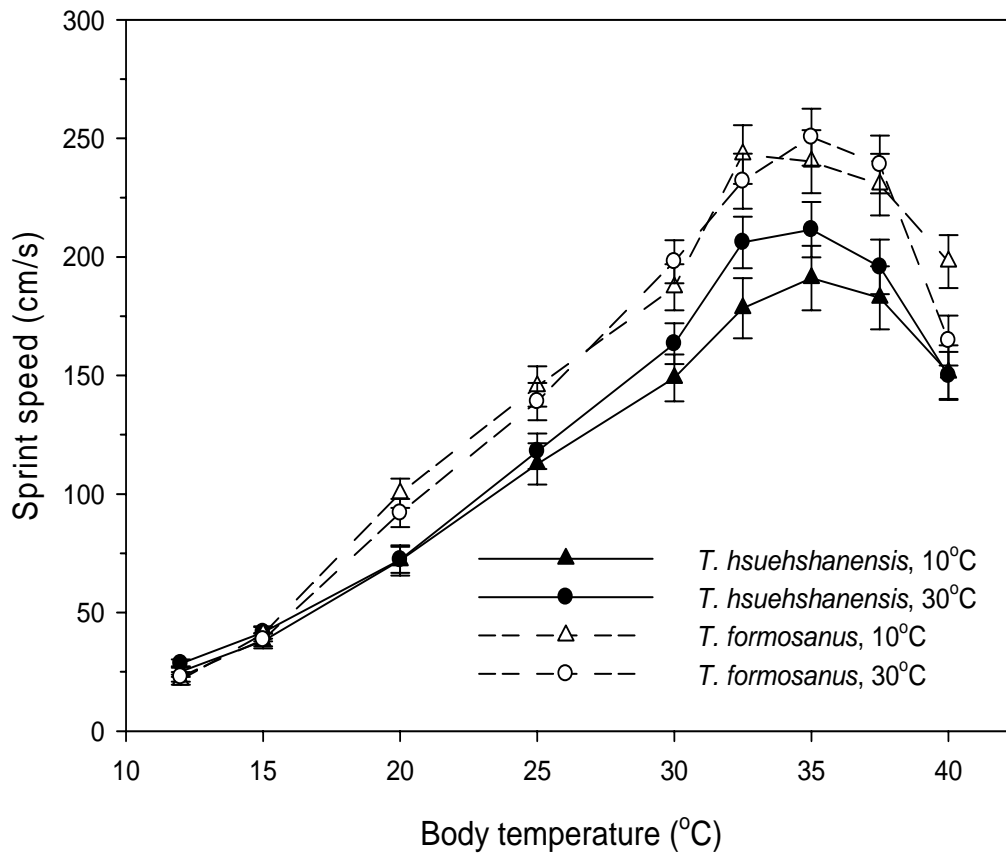


Fig. 2. Absolute sprint speed (estimated mean \pm 1 standard error) of *T. hsuehshanensis* and *T. formosanus* at different body temperatures with two different acclimation temperatures (10°C or 30°C). Sample size: *T. hsuehshanensis*, 10°C, n = 20; 30°C, n = 27; *T. formosanus*, 10°C, n = 21; 30°C, n = 24.

Chapter 5

Effect of lowland environment on the survival rate of high mountainous

Takydromus hsuehshanensis: A transplant experiment study

INTRODUCTION

The distribution of a species is influenced by its geographic barrier, biotic interactions (i.e. competition, predators, parasitism, disease, etc.), and environmental suitability (i.e. temperature, humidity, light, etc.) (Krebs 2001). Understanding the factors that determine a species' distribution is a central objective in ecology (e.g. Caughley et al. 1988, Lawton et al. 1994, Brown et al. 1996, Brown and Lomolino 1998, Krebs 2001) and evolutionary biology (Jablonski 1987). The role of environmental factors is acknowledged to have the potential to affect the geographic distribution range and abundance of a species (Dobzhansky 1950, MacArthur 1972). Examination of the relative influence of environmental factors from the biotic factors on geographic ranges is particularly important because it can provide important applications in predicting species distributions in response to environmental change (Holt 2003) and further species conservation (Wikelski and Cooke 2006).

Two distinct methods are primarily employed to evaluate the mechanistic influence of environmental factors on species distribution ranges (Krebs 2001). One is the evaluation a species' preference range and tolerance limit to a particular environmental factor (such as temperature, humidity, etc.) from the physiological ecology perspective. By comparing the performance/preference ranges and tolerance limits with the recording ranges of environmental factors that occur in the field, I am able to examine the role of an

environmental factor on a species' distribution ranges. For example, environmental temperatures were found to limit the geographic distribution in some ectotherms (amphibians: Wei and Hou 2004; lizards: Heatwole et al. 1969, Gorman and Hillman 1977, Huang et al. 2006).

However, when we try to apply these laboratory measured data to the real world, we should be very cautious in making a conclusion. In the real world, species' geographic ranges may be influenced by interactions of several environmental factors or by some unpredictable and rare climate events (such as extreme heat, cold, or heavy rainfall, etc) that the laboratory treatment is not able to simulate appropriately. Also, a number of researches have shown that a species' tolerance to a factor depends on the levels of another environmental factor. In addition, animals are mobile and will adopt different ways to escape from the harmful environment. Therefore, there are still some limitations in using the laboratory data to interpret the animals' performance in the real world.

The transplant experiment (also known as a common garden experiment, although this term is more plant specific) is an approach that evaluates whether a species is able to live well in "real environmental" conditions in an area that is beyond its native living area. The transplant experiment design where one or more organisms are transplanted from one environment to another (Molles 2002) could eliminate the potential migration limitation of species' geographic ranges, and it also diminishes the influences of biotic interactions if designed properly.

The aim of this study is to investigate the influence of environmental factors on the distribution of *Takydromus hsuehshanensis* using the transplant approach. *T. hsuehshanensis* is the only one species of *Takydromus* lizards that is able to dwell in high

mountain areas (altitude>1800m) in Taiwan. Here, I moved *T. hsuehshanensis* to potential habitats in lowland areas where its lowland closely related species inhabits in. *T. hsuehshanensis* were maintained in semi-natural outdoor enclosures (9 square meters in area). In the enclosures, they were exposed to lowland environments but were able to move freely to perform thermoregulatory behaviours. Plenty of food, water, and shelters (stone, wood, and vegetation) were provided, and potential impacts of predators were deprived.

Chen (2007) had examined suitability of lowland environment for juvenile and embryo of *T. hsuehshanensis*. He conducted a transplant experiment on gravid *T. hsuehshanensis* females using the same outdoor enclosures as I did in this study. In his study, the gravid *T. hsuehshanensis* were moved to the lowland outdoor enclosures, and their embryo and juveniles were maintained in the outdoor enclosures to monitor the survival status. He found that the gravid *T. hsuehshanensis* females were able to oviposit their eggs, and their eggs were able to hatch. However, the juveniles failed to survive over 3 months in the outdoor enclosures. In this study, I focused on adult stage of *T. hsuehshanensis*. I monitored the survivorship, reproduction status and body weight change over 1 year period to investigate whether the lowland was a suitable environment for *T. hsuehshanensis*. Its lowland closely related species *T. formosanus* (currently revised as *T. viridipunctatus*, Lue and Lin 2008) served as a comparison group. I predicted that *T. hsuehshanensis* could not survive well and would gradually lose its body weight over time; on the other hand, *T. formosanus* would maintain a reasonable survival rate and body weight condition.

Due to the large space required to set the outdoor enclosures, I was not able to

construct replicas at other locations in lowland areas. Therefore, inferences drawn from the results of this study, in a restricted sense, were necessarily restricted to the sites I used (the outdoor field of Taipei City Zoo). I did not claim that this site is representative of all areas where lowland *Takydromus* lizards occur in Taiwan. Nevertheless, many lowland *Takydromus* species do live in this site. I therefore assumed that this location was an excellent site for performing the transplant experiment on *T. hsuehshanensis*.

MATERIALS AND METHODS

I collected *T. hsuehshanensis* (males: mass \pm 1SE = 4.7 ± 0.1 g, n=16; females: 4.2 ± 0.1 g, n = 16) from Mt. Hehuan (2400 ~ 3200m in altitude, Nantou County) in May of 2005. During the same time, I also collected *T. formosanus* (mass \pm 1SE = 2.4 ± 0.1 g, n = 16; females: 2.2 ± 0.1 g, n = 16) from Taipei county.

Outdoor enclosures

The transplant experiments were performed in the field at the Taipei City Zoo in a lowland area from late July 2005 to July 2006. The experimental site was located between a forest and a building, with some grasses and bushes growing in it. It was about 60 m in length and 10 m in width, where the lowland *Takydromus* species (*T. stejnegeri*, *T. formosanus* and *T. kuehnei*), and skinks (*Sphenomorphus indicus* and *Eumeces elegans*) lived. I built 8 iron-made outdoor enclosures (width \times length \times height = 3 m \times 3 m \times 0.7 m) contingently, about 0.5 m apart from each other. To prevent an animal's escape from the enclosures, I set the walls of each enclosure 10 cm deep into the ground and folded the tops of the walls 20 cm inwards. A lid made of plastic mesh (pores = 1 cm in diameter) was used to cover the enclosures to prevent predators and human disturbance (Fig. 1).

Inside each enclosure, the vegetation had covered about two-thirds of the areas. I placed several stones ($n = 8$, each larger than 30 cm in diameter) and logs ($n = 6\sim 8$) inside that served as the lizards' shelters. Two thermal data loggers were used to record the temperature underneath the stones and the air temperature (StrowAway, Onset Computer Co., Pocasset, Massachusetts). The difference of the temperatures under the stone among the enclosures was smaller than 2 °C.

Transplanted experiments

Before I transferred the lizards into these semi-natural enclosures, they were acclimated in the thermal incubator of 30 °C which is similar to summer temperature (~ mean summer temperature in the lowland areas, Central Weather Bureau, 1971-2000) for 2 weeks in order to minimize the impacts of immediate exposure to the lowland temperature. In late July of 2005, the tested lizards were put into each outdoor enclosure (8 individuals for each enclosure). I placed females and males in different enclosures to remove the confounding reproduction effect on survival rate. Different species were also put in separate enclosures to prevent interspecies competitions. The individuals were marked by toe-clipping (Hero, 1989). I provided them with enough crickets as food regularly (summer: every 3~4 days; winter: every 3 weeks). I removed any ants' nesting once I found them in the enclosures. These lizards were also detected to eat wild insects inside the enclosures. Water was provided *ad lib* in two water containers (50 L). I measured their body weight and survival state weekly. I also recorded the reproductive status (pregnant or not) and other apparent changes over one year.

Data analysis

Kaplan-Meier survivorship analysis was used to test the effects of 'gender' and

'species' on the survival curves. I assigned the data as "censored" when the tested lizards died due to human-made incidents (*T. hsuehshanensis*: n= 2, *T. formosanus*: n = 2) or their dead bodies were not found in the enclosures (*T. formosanus*: n = 2). Other than the survival rate analysis, I also used Fisher's exact test to analyze the survival rate differences between these two species at 4 time periods during the experiment: the 3rd month (the 13th week), the 6th month (the 26 weeks), the 9th month (the 39 weeks), and the 12th month (the 53th week) after they were put in the enclosures. Percent of body weight change was calculated according to the following equation: Percent of body weight change (%) = 100 % × (body weight_{the nth week} - body weight_{initial}) / (body weight_{initial}). Since females' body weights were dramatically affected by pregnancy and oviposition, they were not included for analysis of results. All statistical analysis was performed with SPSS version 13.0.

RESULTS

The survival rates of both species decreased gradually over time (Fig 2). The gender effect on the survival curves was not significant (*T. hsuehshanensis*: p = 0.62; *T. formosanus*: p = 0.07). After controlling the gender effect, survival curves of these two species differed significantly between each other (p= 0.001, log-rank test). The predicted cumulative survival time for 50% of the individuals was 32 weeks for *T. hsuehshanensis*, but this value could not be estimated for *T. formosanus* because its survival rate was higher than 50% for over a year.

Since survival rates of both species did not differ between females and males (p>0.05) at any of the 4 time periods (the 3rd, 6th, 9th and 12th month), I combined the data

collected from females and males for further analysis. Table 2 shows the survival rates at 4 different time periods. At the 3rd and the 6th month, *T. hsuehshanensis* had survival rates of 80.6 % and 61.2% respectively, which were not significantly different from those of *T. formosanus* ($p > 0.05$). However, at the 9th and the 12th month, survival rates of *T. hsuehshanensis* dropped to only 41.9% and 19.3%, respectively, both of which were significantly lower than the corresponding values of *T. formosanus* ($p < 0.05$) (Table 2).

Figure 3 shows the body weight changes of these two species. *T. hsuehshanensis* gradually increased their body weights after they were translocated into the enclosures. Their body weights reached a plateau at the 5th week in the summer time about an 18% increase of their original body weights (median=18.4%, range: -2.8% ~ 45.0%, n=14). On the other hand, *T. formosanus* increased about 45% of their original body weights (median=44.9%, range: 17.3% ~ 86.9%, n=15) at the 9th week. Since the survival rate decreased over time and thus the sample size was small thereafter, I did not analyze the differences of the weight change between these two species. For *T. hsuehshanensis*, the body weights of individuals one week before death did not drop a lot (median= -2.8%, range= -16.1% ~ 32.3%, n=12). Only two of the dead individuals had body weight less than 90% of their original value.

At the beginning of the experiment, 43.7% of female *T. hsuehshanensis* (n=7) were pregnant. Six females were able to oviposit inside the enclosures, but one of them kept retaining embryos (or yolks) inside its body until it died on the 52nd week. Two eggs of a clutch hatched successfully in October of the first year. During this period, the rain from three typhoons caused the other eggs to be buried under a layer of mud and disappeared. About 50% (n=8) of female *T. hsuehshanensis* were able to survive through the winter.

Among the survivors, 37.5% (n=3) were able to start vitellogenesis in the next year and oviposit unfertilized eggs (n=1~2 for each female) in the summer. Survivors that did not oviposit unfertilized eggs also did not survive to the end of the experiment. On the other hand, all gravid *T. formosanus* females (n=16) were detected to have oviposited, and a total of 24 juveniles were found in the enclosures in the first year. Eight out of 10 survivors that survived winter oviposited unfertilized eggs in the next year..

Noticeably, in the winter (December 2005 to February 2006) *T. hsuehshanensis* was observed to be more active and to bask more frequently than did *T. formosanus*, but they appeared to be unhealthy. For example, a portion of them had skin dried up and accumulated on their body, and only bits and pieces came off causing lots of old skin to remain on the body (males: n=7 over 10 individuals, females: n=4 over 10 individuals); *T. formosanus* did not show any signs of skin shedding. In addition, some individuals of *T. hsuehshanensis* had some problems in their toes. Their toes of some individuals (males: n=7 over 10 individuals, females: n=7 over 10 individuals) festered, and consequently lost the tips of these toes for unknown reasons. *T. formosanus* had significantly less individuals having this illness (males: none over 14 individuals; female: 2 over 11 individuals) ($p < 0.0001$, Fisher's exact test). In addition, the eyes of some *T. hsuehshanensis* individuals (male: n=1; female: n=1) became swollen, reddish and completely closed, which consequently caused them to be unable to hunt and to eventually die. Only one *T. formosanus* individual had this eye problem, but it gradually recovered in 2 weeks. I did not find any visible external parasites on the skin of both species.

DISCUSSION

The results indicated that *T. hsuehshanensis* was not able to survive well in the outdoor enclosures in the lowland area over a year. Since the juveniles of the *T. hsuehshanensis* that had hatched in the outdoor enclosures were not able to survive over 3 months in the outdoor enclosures (Chen, 2007), I supposed that it would be a hardship for *T. hsuehshanensis* to build a population in the lowland area in their current state.

However, since a few *T. hsuehshanensis* individuals were still able to survive and some females may have potential reproductive abilities, the environmental factors in the lowland area appeared to be livable for a few individuals. According to Holt (2003), “A species' distribution limits may shift in space either because of changes in ecological factors (e.g. climate, the abundances of interacting species) or because the traits that influence range limits (e.g. dispersal, niche characteristics) evolve by natural selection”. Therefore, I would not exclude the possibility that some *T. hsuehshanensis* individuals could gradually evolve some traits that enable them to survive well in the lowland environment if they were to disperse toward the lowland areas.

According to the results, I assumed that the winter environment in the lowland areas may have caused a negative effect on adult *T. hsuehshanensis*. *T. hsuehshanensis* did not grow well and shed their skin abnormally, whereas lowland dormant *T. formosanus* remained healthy. Compared to the dormant *T. formosanus*, *T. hsuehshanensis* were more active, implying that hibernation may be required for *T. hsuehshanensis* to maintain its normal, year-round physiological functions. Actually, in the winter in its native mountain habitats, *T. hsuehshanensis* would hide underground to hibernate throughout the cold weather. According to previously studies, some abnormal physiological functions, such as dysfunction of the thyroid gland (Rivera and Lock 2008) and disruption of the

reproduction cycles (Naulleau 1970) were detected in reptiles that did not enter proper hibernation. Also, it has also been proposed that hibernation strategy adopt by many ectotherms would decrease energy expenditure during winter (Lillywhite 1987). Whether hibernation was needed for maintain *T. hsuehshanensis*' year-round normal function remains unclear. Further studies regarding examination the role of hibernation on *T. hsuehshanensis*, such as inspection the healthy states of hibernation-deprived treated *T. hsuehshanensis*, will help confirm my suggestion.

Environmental temperature was proposed as a cue for reptiles to enter and to terminate from a hibernation state (Jacob and Painter 1980, Sanders and Jacob 1981; review: Gregory 1982). In this study, I found the winter temperature of the outdoor enclosures was not low (mean=15.8°C), and it spanned across a large range (range=6.9~27.2 °C). I suggested that the lowland thermal environment was probably higher than upper limit of the body temperatures range that *T. hsuehshanensis* normally had during its hibernation state; however, it may not be high enough for *T. hsuehshanensis* to perform normal physiological functions. Further investigations regarding thermal biology, particularly the preferred temperature of *T. hsuehshanensis* in different seasons, will help to clarify this suggestion.

In contrast to winter, it was clear that *T. hsuehshanensis* were able to survive well and to increase body weight during the summer. Moreover, they were able to shed their skin regularly, and the spot color on the males' dorsal lateral body was a bright yellow, both of which were healthy signs for lizards. Similar results were found in the repeated experiment during the next summer. Therefore, summer did not seem to be a critical period for *T. hsuehshanensis* to survive in lowland areas.

Other than the temperature, abiotic factors (i.e. the humidity, rainfall, and etc) in lowland areas also differed a lot from that in high altitude, and all of these variables could be the reason causing the low survival rate of *T. hsuehshanensis* that maintained in outdoor enclosure in lowland areas. Another explanation for the low survival rates of *T. hsuehshanensis* in lowland areas could be due to habitat unsuitability inside outdoor-enclosures. Further investigations are required to confirm these possibilities.

The mechanistic reason for high mortality of *T. hsuehshanensis* could be a greatly reduced immune system function, instead of negative balance in energy budget. According to the results, most of the *T. hsuehshanensis* individuals were able to retain a reasonable body weight before they died. Nevertheless, they were not healthy during the transplanted period even though they were given enough food. Many of them had festering toes, eye diseases, or unhealthy skin shedding before they died. Some of these symptoms could be caused by viral and/or bacterial infection, implying that some infectant micro-organisms may be also causing the low survival rate of *T. hsuehshanensis*. Further pathological investigations on these unhealthy individuals would help confirm my interpretation.

In summary, I found that most *T. hsuehshanensis* individuals did not survive well in lowland areas, but a few of them were able to survive well for one year. I supposed in the winter, the lowland areas may cause more severe negative impact on year-round successful survive of *T. hsuehshanensis* in lowland areas. Other than the abiotic factors, some microorganism-related infection may also cause the low survival of *T. hsuehshanensis*. Therefore, not only abiotic factors, but also the microorganism infections would be worth further to work on investigating current altitudinal distribution

of adult *T. hsuehshanensis*. As mentioned by Krebs (2001), “environmental factors may act on any stage of the life cycle and can limit the distribution of a species through their effect on one or more of the following: survival, reproduction, development of young organisms, and interactions (competition, predation, parasitism, and diseases) near the limit of tolerance”. Further studies that cover different stages of the life cycle, concerning different aspects, such as the interaction with other organisms, on transplanted *T. hsuehshanensis* in lowland areas would help to clarify the environmental effect on *T. hsuehshanensis*’ current altitudinal ranges.

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Table 1. The temperature recordings in the air and underneath stone in the outdoor enclosures.

Recordings	Air temperature (°C)	Temperature underneath stone (°C)
<i>The year 2005</i>		
Summer		
Mean temperature	25.8	27.0
Maximum temperature	35.5	39.0
Minimum temperature	21.4	22.2
Recording period	7/23~8/31	7/23-8/31
Date of missing data	8/8-8/13	8/5-8/10
Autumn		
Mean temperature	22.9	22.3
Maximum temperature	37.1	34.5
Minimum temperature	16.5	17.0
Recording period	9/1-11/31	9/1-11/31
Missing data (duration)	no	9/1-6; 10/12-14
Winter		
Mean temperature	15.8	16.0
Maximum temperature	27.2	27.9
Minimum temperature	6.9	8.2
Recording period	12/1-2/28	12/1-2/28
Missing data (duration)	2/8-2/28	no
<i>The year 2006</i>		
Spring		
Mean temperature	22.7	20.2
Maximum temperature	35.8	32.3
Minimum temperature	16.3	10.7
Recording period	3/1-5/31	3/1-5/31
Missing data (duration)	3/1-4/13	no
Summer		
Mean temperature	22.7	25.8
Maximum temperature	35.8	31.4
Minimum temperature	19.0	20.2
Recording period	6/1-7/23	6/1-7/23
Missing data (duration)	no	no

Table 2. The survival rate of *T. hsuehshanensis* and *T. formosanus* in different time periods

species	13 weeks (3rd month)	26 weeks (6 th month)	39 weeks (9 th month)	53 weeks (12 th month)
<i>T. hsuehshanensis</i>	80.6 %	61.2 %	41.9 % ^a	19.3 % ^a
<i>T. formosanus</i>	83.8 %	83.8 %	80.6% ^b	80.6% ^b
<i>p</i> value	0.31	0.08	0.003*	<0.0001*

* different letters indicate significant different in survival rates in these two species using Fisher's exact test.



Fig. 1. Outdoor enclosures. The outdoor enclosures were iron-made (width \times length \times height = 3 m \times 3m \times 0.7 m). The button sides of the walls in each enclosure were set underground into 10cm deep, and their upper sides were folded 20 cm inside. The lid made of plastic mesh (pores = 1 cm in diameter) was used to cover the enclosures to prevent predators and human disturbance and to prevent animal's escape from the enclosures.

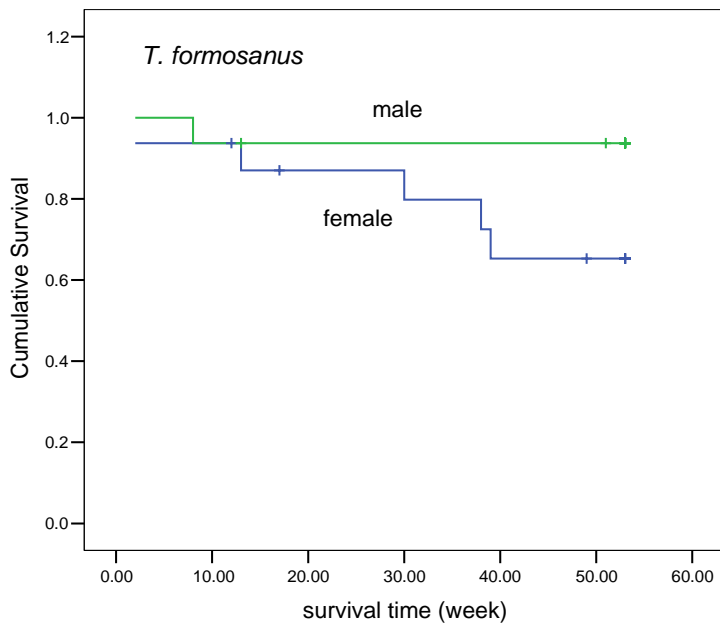
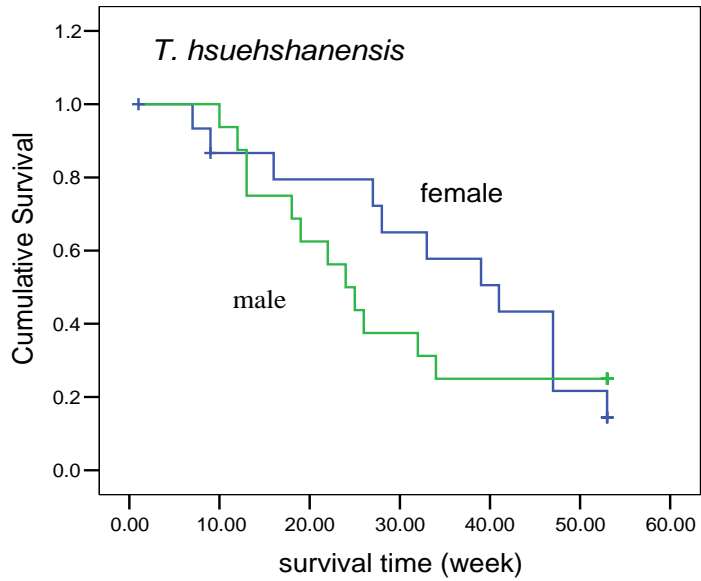


Fig. 2. The survival curves of *T. hsuehshanensis* (A) and *T. formosanus* (B). Sample size for both female and male in each species was 16.

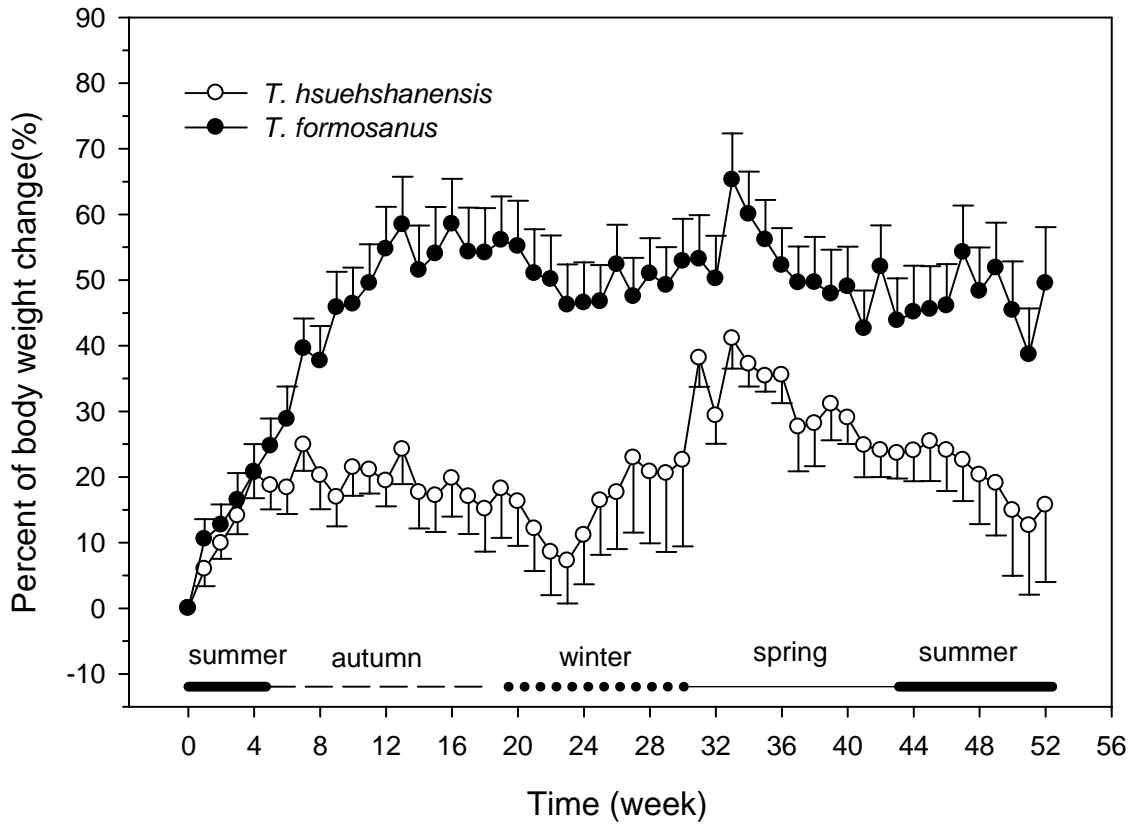


Fig. 3. The body weight change of *T. hsuehshanensis* (mean - 1SD) and *T. formosanus* (mean + 1SD) over one year period. Percent of body weight change (%) = $100 \% \times (\text{body weight}_{\text{the nth week}} - \text{body weight}_{\text{initial}}) / (\text{body weight}_{\text{initial}})$. Note: the sample size varies over time because the survival rate decreases over time. Sample size: *T. hsuehshanensis*: n=14 at the beginning, and n=3 at the end; *T. formosanus*: n=14 at the beginning, and n=11 at the end.

Chapter 6

Conclusion

Comparing the cold tolerances and heat tolerances of *T. hsuehshanensis*, *T. formosanus*, and *T. stejnegeri* with the temperature recordings from the weather station and the spots I set in the wild, I have demonstrated that neither cold tolerance nor heat tolerance was the crucial physiological constraints that caused the current altitudinal distributions of these three species. I also detected that cold tolerance was positively correlated to the upper limits of their altitudinal distribution, implying that the cold temperature may form as a strong natural force selecting cold tolerance of these species. As for the locomotor performance, the sprint speed of *T. hsuehshanensis* was not reduced in the range of the normal summer air temperature range in the lowland areas, showing that temperature was not a physiological constraint affecting the current altitudinal distribution of *T. hsuehshanensis*. The thermal-dependence of the sprint speed was not significantly different between *T. hsuehshanensis* and *T. formosanus*, indicating that the varied thermal environments did not force these two species to differentiate their thermal sensitivity in the locomotor performance. The *T. hsuehshanensis* was not able to survive well in lowland outdoor enclosures for one year. Either the climate of the environment or microorganism infections may explain its low survival rate. However, since the transplant experiment only lasted for only one year, further investigations are needed to clarify this interpretation.