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Differences in thermal biology between two morphologically distinct populations of Iberian wall lizards inhabiting different environments

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Populations should adapt to the climate at their respective localities. Here, we examined differences in thermal biology between two populations of *Podarcis hispanica* lizards from areas with different climates. Lizards from the cold, northern mountains attained lower field body temperatures than lizards form the warm, southern plains. However, the larger body size and darker coloration of northern lizards resulted in slower cooling rates, which may increase efficiency of thermoregulation. Northern populations selected higher temperatures in a thermal gradient, possibly as an adaptation to heat up before conditions changed, as their mountain environment is unpredictable. Finally, lizards from both populations had similar temperature-dependent locomotor performance curves, although southern lizards were relatively faster considering their smaller body size. We suggest that environmental differences may lead to differences in morphology and locally adapted thermal biology in lizards that might allow maximizing thermoregulation in each local climatic conditions.

Introduction

Alternative environments across geographical space can result in different phenotypic traits conferring high fitness in different areas (Endler 1977, Schluter 2001). This may result in a mosaic of locally-adapted populations, and such variation may play an important role in evolutionary diversification (e.g., Badyaev *et al*. 2000). In lizards, an association between habitat use, body shape and performance has frequently been found (e.g., Losos 1990, Melville & Swain 2000, Herrel *et al*. 2002, Elstrott & Irschick 2004). Habitat, broadly defined, is an important factor that may influence behavior, performance, and even morphology (Huey 1991). Temperature is a habitat characteristic that may affect performance indirectly through its effect on physiological processes, and directly by affecting behavior (Huey 1982, Arnold & Bennett 1984, Van Damme *et al*. 1990, 1991), including locomotor performance (Bennett 1990, Garland 1994, Bauwens *et al*. 1995, Du *et al*. 2000). For example, the body temperature of an ectotherm may affect its ability to avoid predators (Christian & Tracy 1981), and individual differences in thermoregulatory efficacy may be crucial for survival (Waldschmitd & Tracy 1983).

The performance of lizards and other ectotherms depends largely on their body temperature, which is primarily a function of environmental conditions and thermoregulatory behavior (Huey & Kingsolver 1989, Angilletta 2009). The ability of an individual to attain and maintain body temperatures suitable for activity is also dependent on its phenotypic properties, such as body size and coloration (Digby 1955, Willmer & Unwin 1981, Heinrich 1996). Heating capacity is affected by body size and coloration. Larger individuals heat up more slowly but achieve greater temperature excess than smaller ones (Digby 1955, Stevenson 1985, Carrascal *et al*. 1992, Heinrich 1996). Darker individuals attain higher steady-state body temperatures than light-colored phenotypes (De Jong *et al*. 1996, Bittner *et al*. 2002, Forsman *et al*. 2002, Clusella-Trullas *et al*. 2007, 2009). We could expect that populations living in different environments evolve morphologies and coloration adapted to the local climatic conditions.

Wall lizards' species (*Podarcis* spp.) are the predominant lizard group in southern Europe. Their taxonomy is complex and unstable, primarily because species exhibit substantial levels of intraspecific morphological variation (Arnold & Burton 1978). Molecular and morphological studies suggest that the Iberian wall lizard, *Podarcis hispanica*, is paraphyletic, and forms part of a species complex with at least five monophyletic lineages (Guillaume 1987, Harris & Sá-Sousa 2001, 2002, Sá-Sousa *et al*. 2002, Pinho *et al*. 2007, Carretero 2008). In the western and central parts of the Iberian Peninsula, two morphotypes have been described. In northwestern Iberia, *P. hispanica* type 1 occurs, mainly in highlands and where Atlantic humid environmental conditions prevail, while *P. hispanica* type 2 occurs in central and southern Iberia, where Mediterranean dry conditions are typical (Sá-Sousa 2000, Sá-Sousa *et al*. 2002). These two morphotypes also differ genetically, corresponding with two of the described genetic lineages (Pinho *et al*. 2007, Carretero 2008). Both lineages/types have been reported from the Madrid Region (Central Spain) occupying very different environments (García-Paris *et al*. 1989, Martín & López 2006a, 2006b, Gabirot *et al*. 2012). Morphological characteristics may allow to assign lizards from the mountains north of Madrid to the previously described morphotype 1 of *P. hispanica*, whereas lizards from the plains south of Madrid are similar to the morphotype 2 (Guillaume 1987, Sá-Sousa 2000, Sá-Sousa *et al*. 2002).

The questions that arise are (1) whether differences between these morphotypes of *P. hispanica* may be an adaptation to the environmental conditions of the habitat currently occupied by each population, which could match the conditions occurring in the past in their biogeographical area of origin (Sá-Sousa 2000, Carretero 2008). (2) Are the optimal temperatures for activity of lizards correlated with climatic conditions in their respective habitats? And (3) do individuals from all populations select the same optimal temperature for activity in spite of differences in local environments?

The preferred body temperature (T_p) is important because it is correlated with the optimal temperature of many physiological processes (Huey & Bennet 1987, Bauwens *et al*. 1995, Angilletta *et al*. 2002, Angilletta 2009). However, T_p is considered to be "evolutionarily rigid" in lacertid lizards because it changes very slowly in response to long-term changes in the thermal environment (Van Damme *et al*. 1990). Thus, there is variation in T_p between species (Bauwens *et al*. 1995), but T_p usually remains similar between different populations of the same species inhabiting different environmental conditions (Van Damme *et al*. 1989, 1990, Gvozdík & Castilla 2001, Carretero *et al*. 2005). Nevertheless, some studies show that different "forms" within the *P. hispanica* species complex select different temperatures (Carretero *et al*. 2006, Veríssimo & Carretero 2009), suggesting that these forms are separated by independent evolution which produced changes in this otherwise conservative physiological trait.

In this paper, we examined the potential differences in thermal biology between two populations of *P. hispanica* lizards from central Spain with distinct morphotypes. We selected two populations (northern mountains *vs*. southern plains) living in different climatic conditions. We first characterized morphology and dorsal coloration of lizards, and examined experimentally the heating and cooling rates of lizards from both populations. Then, we described climatic conditions of the two populations, measured field body temperatures of lizards in their natural populations, and measured selected body temperatures of lizards in a laboratory thermal gradient. Finally, we tested the locomotor performance (fleeing speed) of lizards at different temperatures to examine the degree of adjustment between thermal biology and physiological performance in each population. If the optimal temperature for performance is affected by local environmental conditions it should differ between populations (i.e., being lower in the northern population). We hypothesized that differences in temperatures could have lead to differences in morphology and thermal biology between these lizard populations as an adaptation to different habitats.

Material and methods

Animals and study areas

During March 2009, we captured *P. hispanica* lizards (n = 17; 10 males and 7 females) from a population occupying granite rock-cliffs at the edge of a pine forest in the upper part of the Fuenfría Valley (40°47´N, 04°03´W; 1750 m a.m.s.l.). We also captured *P. hispanica* lizards (n = 24; 15 males and 9 females) on chalk and gypsum rocks in deforested bushy hills south of Madrid, near the village of Aranjuez (40°02´N, 03°37´W; 494 m a.m.s.l.). These two *P. hispanica* populations could be assigned to the previously-described morphotypes 1 and 2, respectively (Sá-Sousa *et al*. 2002).

All lizards were housed individually at the El Ventorrillo Field Station (Cercedilla, Madrid) about 5 km from the Fuenfría population, in indoor 60 × 40 cm PVC terraria containing sand substratum and rocks for cover. Cages were heated with 40 W spotlights for 6 hours a day, and illuminated by overhead 36 W full-spectrum daylight tubes (10 h:14 h light/dark cycle). They were also screened from each other using cardboard. Every day, lizards were fed mealworm larvae (*Tenebrio molitor*) dusted with multivitamin powder for reptiles, and water was provided *ad libitum*. At the end of the studies, the lizards were returned to their exact capture sites in good condition.

Environmental climatic conditions

We summarized the available climatic conditions in each lizard population by using data from two meteorological stations located close to each lizard population, at the same altitude and in the same type of habitat. These stations have recorded temperatures, relative humidity, precipitation and wind speed every day for at least 10 years (data available at http://www.tutiempo.net/clima). We used data from the meteorological station of Navacerrada, located in the mountains north of Madrid, 5 km from the Fuenfría lizard population, and the southern station located in Toledo, 30 km from the Aranjuez lizard population. We used Student's t-test to test for differences in weather variables between the stations. We used data from the period March to August as this is the season when the lizards are most active. Lizards are either little active or hibernating during the rest of the year.

Morphology and coloration of lizards

We measured the snout-to-vent length (SVL) of each captured lizard with a ruler (to the nearest 1 mm) and the body weight with a digital balance (to the nearest 0.1 g). We calculated the body condition index (BCI) as the residual from the regression of body weight on SLV, both ln-transformed (lineal regression: $r = 0.86$, $F_{1,39} = 108.88$, $p < 0.0001$). We used two-way ANOVA to analyze differences in morphological variables (log-transformed) between populations and between sexes, including the interaction between sexes and populations in the models.

We measured reflectance of lizards' dorsal coloration from 350 to 700 nm using an Ocean Optics USB2000 spectroradiometer with a DT-1000-MINI Deuterium–Halogen light source (Ocean Optics, Inc., Dunedin, FL). To exclude ambient light and standardize measuring distance, a cylindrical metallic tube was mounted on the bifurcated fiber optic probe (Montgomerie 2006). The probe was held at a 90° angle to the skin, and reflectance was measured, always by the same person (MG). We measured coloration of two standardized spots on the dorsum of the lizards: one in the middle between the two forelimbs, and one in the middle between the hind limbs. Reflectance was calculated relative to a white standard (WS-1-SS) with the OOIBase32 software (Ocean Optics, Inc.). We transformed reflectance values following formulas described in Montgomerie (2006) to calculate mean brightness and hue of dorsal coloration, and then calculated a mean of the two measurements for each lizard.

Field body temperatures

During the spring, we captured active lizards from the two populations by noosing, and immediately measured their field body temperatures (T_{b}) (to the nearest 0.1 °C) by inserting the probe of a digital thermometer (Quartz digi-thermo) into the cloaca. All temperatures were taken during days when environmental temperature and sunny conditions allowed lizards to be fully active and during the late morning (between 11.00 and 13:00) when lizard activity was maximal. We expected that measured T_{b}s reflected those that lizards were able to attain and maintain under favorable conditions. In fact, active behavioral thermoregulation allows many lacertid lizards to maintain remarkably constant T_{b} throughout their daily activity period, despite significant variability in environmental temperatures (Bauwens *et al*. 1996, Castilla *et al*. 1999). We used two-way ANOVA to compare T_{b}s between populations and between sexes, including their interaction in the model.

Selected body temperatures

We created a thermal gradient in an indoor 100 × 50 × 50 cm glass terrarium by suspending three light bulbs (25 W, 60 W and 250 W) 22 cm above the terrarium's floor. Two bulbs (25 and 250 W) were placed 15 cm from the shorter terrarium walls, and the 60 W bulb was placed in the center of the terrarium. Thus, we created a gradient of air temperatures ranging from 15 to 53 °C. Inside the terrarium, there was a vermiculite substrate to facilitate lizards' traction, but we did not place any rock, feeder, etc. to avoid disturbing the thermal selection tests. The lights were switched on 1 h before trials to establish a stable thermal gradient. We also recorded the air temperature of the room at the beginning and the end of the experiments to ensure that conditions were similar (average air temperature: 14.3 ± 0.2 °C) through all experiments.

We took one lizard from its home terrarium, where it was maintained at a relatively low temperature (initial body temperature of lizards was 15.1 ± 0.1 °C), introduced it gently into the thermal-gradient terrarium, and let it stay there for 1 h to allow it to choose a location where it could attain a preferred body temperature. To measure selected body temperatures (T_{sel}; to the nearest 0.1 °C) of the lizards by insterting the probe of a digital thermometer (Quartz digi-thermo) into the lizard cloaca to record, we gently removed them for a short while 30 min and 60 min after introducing them to the thermal gradient. For each lizard from the two populations we repeated the experiment twice, on two different days and in a random order. Then, we calculated an average T_{sel} for each lizard from the four measurements. These readings were considered estimates of the behaviorally-preferred body temperatures, and we assumed that they represented the body temperatures that lizards would maintain in the absence of abiotic and biotic restriction (Licht *et al*. 1966). To avoid bias in the measurement procedure, all temperature measurements were carried out by the same person (AB). Temperatures were log-transformed to obtain a normal distribution, and we used two-way ANOVA to test for differences in T_{sel} between populations and between sexes, including their interaction in the model.

Heating and cooling experiments

We took one lizard from its home terrarium, fixed it to a flat granite rock with two bands of transparent masking tape (around the base of the tail and on top of the forelimbs), and positioned it under a 250 W light bulb placed 38 cm above the animal. At the beginning of the trials, all the lizards had a similar low body temperature (21.6 ± 0.5 °C). To estimate the heating rate of the animal, after switching on the light we measured the body temperature every 5 s with a digital quick-reading thermometer (Quartz digi-thermo) inserted into the lizard's cloaca (precision of 0.1 °C). The heating experiment was stopped when the cloacal temperature reached 32 °C (approx. 12 °C below the critical thermal maximum for medium sized lizards; Bauwens *et al*. 1995). To estimate the cooling rate, immediately after switching off the light, we noted the cloacal temperature every 5 s for 5 min, until it reached approx. 26 °C. Ten individuals (5 males and 5 females) from each population were tested in random order.

We calculated thermal time constants (τ) for heating and cooling rates following the methods of Dzialowski and O'Conner (2001) and Rice *et al*. (2006). We determined the natural logarithm of the difference between the body temperature of each lizard (T_b) and the final equilibrium temperature (T_{eq}). Plotting $\ln(T_b - T_{eq})$ *versus* time yielded a linear relationship. We used the inverse of the slope to determine each thermal time constant (τ). We used two-way ANOVA to compare thermal time constants in the heating or cooling experiments between populations and sexes. Because heating and cooling rates could be affected by the body weight of animals (Carrascal *et al*. 1992, Martín & López 2003), we also analyzed the thermal constant differences between populations with three-way ANOVA including the lizards' body weight as the covariate.

Speed at different temperatures

Fleeing speed was measured by racing a lizard as fast as it could run along an indoor racetrack (120 × 30 × 40 cm) paved with polystyrene that provided excellent traction and with no obstacles. We recorded all races on a videotape (Hi-8 format, 25 frames s^{-1}) using a Sony CCD-V800E video-camera aligned perpendicularly over the center of the terrarium (1.60 m above the ground). We analyzed the maximum fleeing speed of lizards at six test temperatures (15, 20, 25, 30, 35, and 37.5 °C). Body temperature of lizards prior to testing was adjusted by placing them in an incubator chamber (Friocell, Königswinter, Germany) at one of the test temperatures for at least 30 min. Then, the animal was removed from the incubator, its body temperature was measured with a thermometer to ensure that it attained the T_b for that treatment, after which it was gently placed in the racetrack. A lizard was stimulated to run by tapping the substrate with a stick close to the end of its tail. Individuals were tested once at each test temperature, but in each trial the lizards were made to run five times. The escape sequences were spaced sufficiently so that fatigue resulting from one run did not affect subsequent runs. Trials at different temperature were performed on different days and in random order to avoid fatigue of the lizards to affect their performance. Ten individuals (5 males and 5 females) from each population were tested that way. All the tested lizards had intact or completely regenerated tails to avoid the effect of different tail lengths on fleeing speed (Martin & Avery 1998).

Recordings were analyzed frame by frame to calculate fleeing speed based on calibrated distances measured (in mm) from the video monitor, using the tip of the snout of the lizard as a reference position. Three runs of each individual in each temperature were analyzed. For each sequence, the distance between the initial position (lizard not moving) of lizard's snout and the final position in the first pause after fleeing (escape distance), and the time interval between the initial and final positions (escape duration) were measured. From these data, the absolute average speed (covered distance divided by ttime; cm s^{-1}) was calculated (Martin & Avery 1998).

Due to differences in body size, smaller lizards have often lower absolute speed than large ones (Martín & López 1995, Irschick 2000). Thus, for each lizard at each test temperature we also calculated the relative average speed (SVL

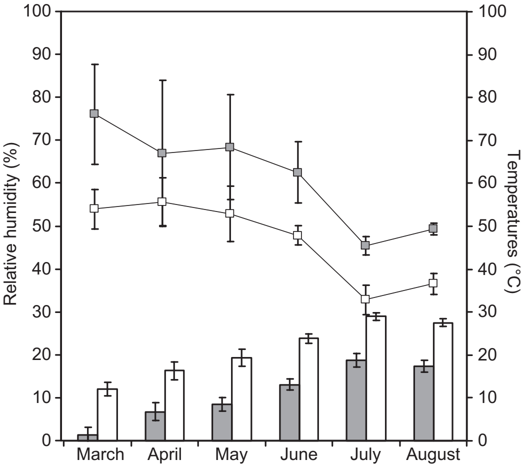

Fig. 1. Climatic characteristics (relative humidity and temperature) recorded by two meteorological stations close to the lizards' capture sites: Navacerrada station (open boxes) was close to the Fuenfría lizard population and Toledo station (black boxes) was close to the Aranjuez lizard population.

s^{-1}) (*see* also Martín & López 1995, Irschick 2000). We used two-way repeated-measures ANOVA to compare absolute and relative average speeds between populations (between factor) and test temperatures (within factor).

Results

Differences in local environments

The climatic data from the Navacerrada (i.e., close to Fuenfría lizard populations) and Toledo meteorological stations (near Aranjuez lizard populations) showed that during the reproductive season of the lizards (from March to August) these areas differed significantly in temperature (*t*-test: $t_{10} = -2.83$, $p = 0.017$) and in relative humidity ($t_{10} = 3.03$, $p = 0.012$) (Fig. 1). The climate in Fuenfría was colder and with more humidity than in Aranjuez. There were no significant differences between the localities in terms of precipitation and wind speed ($p > 0.09$ for both).

Morphology and coloration of lizards

The lizards from Fuenfría were significantly larger and heavier than those from Aranjuez, but in both populations, males were significantly larger and heavier than females. However, we did not find significant differences in BCI between populations or sexes (Tables 1 and 2). Therefore, differences in body weight resulted from differences in body length rather than from differences in body shape.

The lizards from Aranjuez had significantly lighter dorsal coloration than lizards from Fuenfría (mean brightness: 9.6% ± 1.2% *vs*. 3.8% ± 0.3 %, respectively; one-way ANOVA: $F_{1,25} = 29.94$, $p < 0.0001$), but they did not differ significantly in hue of coloration (624.7 ± 14.4 nm *vs*. 637.5 ± 7.9 nm respectively; one-way ANOVA: $F_{1,25} = 0.70$, $p = 0.40$).

Field and selected body temperatures

The lizards from Aranjuez attained significantly higher T_bs in the field than lizards from Fuenfría, and in both populations, males attained significantly higher T_bs than females (Fig. 2a and Table 2). In contrast, lizards from Fuenfría selected significantly higher temperatures in the thermal gradient than lizards from Aranjuez, and males from both populations selected significantly higher temperatures than females (Fig. 2b and Table 2).

Table 1. Mean (± SE) body size (snout-to-vent length; SVL), body weight, and body condition index (BCI) of male and female *P. hispanica* lizards from the two populations (Aranjuez and Fuenfría).

	Aranjuez		Fuenfría	
	Males	Females	Males	Females
Size (SVL) (mm)	51 ± 1	45 ± 1	57 ± 1	55 ± 2
Weight (g)	3.3 ± 0.2	2.5 ± 0.1	5.1 ± 0.2	3.9 ± 0.4
BCI	–0.04 ± 0.04	0.05 ± 0.08	0.08 ± 0.04	–0.04 ± 0.06

Heating and cooling rates

The lizards from Fuenfría heated and cooled significantly slower than lizards from Aranjuez as indicated by the significantly greater time constants (Fig. 3 and Table 2). Similarly in both populations, males heated and cooled significantly slower than females (Fig. 3 and Table 2). In addition, lizards heated significantly faster than they cooled (one-way repeated measures ANOVA: $F_{1.19} = 11.95$, $p = 0.0026$).

Body size clearly affected thermal constants, and when body mass was included in the analyses as the covariate there were no significant differences between populations (three-way ANOVA: heating: $F_{1.15} = 0.27$, $p = 0.61$; cooling: $F_{1.15} = 1.64$, $p = 0.22$) or sexes (heating: $F_{1.15} = 1.44$, $p = 0.25$; cooling: $F_{1.15} = 0.69$, $p = 0.42$), and their interactions were not significant (population × sex, heating: $F_{1.15} = 0.78$, $p = 0.39$; cooling: $F_{1.15} = 0.01$, $p = 0.95$). Therefore, differences between populations and sexes in body mass alone may explain the differences in heating and cooling rates. In fact, when pooling lizards from both populations, body mass was positively and significantly correlated with the thermal constants for heating (lineal regression: $r = 0.63$, $F_{1,18} = 12.05$, $p = 0.003$) and cooling ($r = 0.75$, $F_{1,18} = 23.21$, $p = 0.00014$). Thus, larger lizards heated and cooled slower.

Speed at different temperatures

Temperature significantly affected absolute average speed (cm s^{-1}) of lizards (three-way repeated measures ANOVA: $F_{5,80} = 99.88$, $p < 0.0001$), and in both populations males were significantly faster than females ($F_{1,16} = 9.40$, $p = 0.007$), but no significant differences between populations were found ($F_{1,16} = 0.28$, $p = 0.60$), and none of the interactions were significant ($p > 0.40$ in all cases) (Fig. 4a). In both populations and sexes, absolute average speed was significantly higher at 35 °C (Tukey's tests, $p < 0.004$ in all cases) than at 15 °C ($p = 0.00012$ in all cases), and there were significant differences in speed between the test temperatures ($p = 0.00012$ in

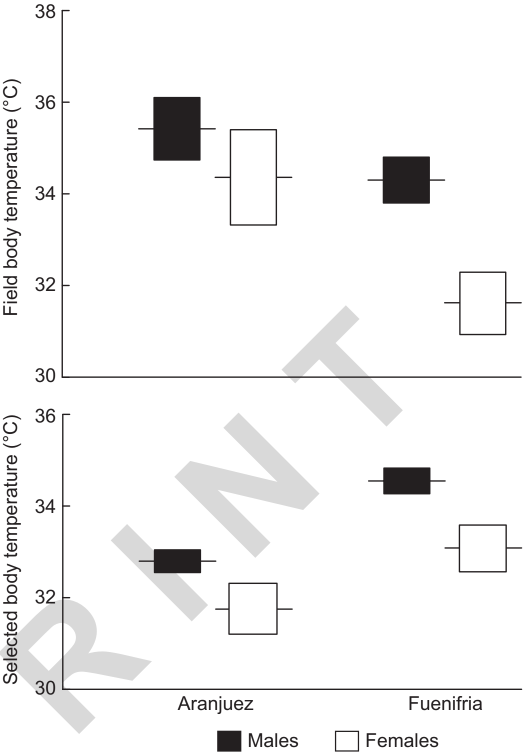

Fig. 2. Mean (± SE) (**a**) field and (**b**) selected body temperatures of male and female *P. hispanica* lizards from the Aranjuez and Fuenfría populations.

Table 2. Differences in morphological and thermal biology variables between populations (Aranjuez and Fuenfría) and between sexes of *P. hispanica* lizards (two-way ANOVA).

	Population	Sex	Population × sex
Size (SVL)	$F_{1,37} = 31.07$, $p < 0.0001$	$F_{1,37} = 8.64$, $p = 0.006$	$F_{1,37} = 1.42$, $p = 0.24$
Weight	$F_{1,37} = 24.27$, $p < 0.0001$	$F_{1,37} = 6.94$, $p = 0.012$	$F_{1,37} = 0.04$, $p = 0.85$
Body condition index	$F_{1,37} = 0.06$, $p = 0.80$	$F_{1,37} = 0.03$, $p = 0.85$	$F_{1,37} = 3.06$, $p = 0.09$
Field body temperature	$F_{1,26} = 6.62$, $p = 0.016$	$F_{1,26} = 6.17$, $p = 0.019$	$F_{1,26} = 1.16$, $p = 0.29$
Selected temperature	$F_{1,35} = 16.80$, $p = 0.0002$	$F_{1,35} = 10.90$, $p = 0.002$	$F_{1,35} = 0.20$, $p = 0.64$
Heating rate	$F_{1,16} = 6.42$, $p = 0.022$	$F_{1,16} = 5.31$, $p = 0.035$	$F_{1,16} = 1.46$, $p = 0.24$
Cooling rate	$F_{1,16} = 15.41$, $p = 0.0012$	$F_{1,16} = 5.12$, $p = 0.038$	$F_{1,16} = 0.18$, $p = 0.68$

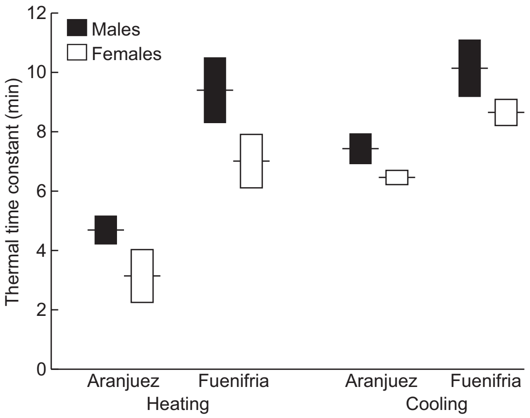

Fig. 3. Mean (± SE) thermal time constant (τ) for heating and cooling of male (black boxes) and female (open boxes) *P. hispanica* lizards from the Aranjuez and Fuenfría populations.

all cases), except between 20 °C and 25 °C (p = 0.99) and between 30 °C and 37.5 °C ($p = 0.10$).

Relative average speed (SVL s^{-1}) was significantly and in a similar way as the absolute one affected by the test temperatures (three-way repeated measures ANOVA: $F_{5,80} = 99.88$, $p <$ 0.0001). Also, there were no significant differences between sexes ($F_{1,16} = 3.07$, $p = 0.10$), but there were significant differences between populations ($F_{1,16} = 5.77$, $p = 0.029$), with lizards from Aranjuez being relatively faster, considering their smaller body size, than lizards from Fuenfría (Fig. 4b). The effect of temperature on relative speed was similar in both populations (interaction temperature × population: $F_{5,80}$ = 1.09, $p = 0.37$), and in both sexes (interaction temperature × sex: $F_{5,80} = 0.46$, $p = 0.80$), all the other interactions being insignificant ($p > 0.90$ in all cases).

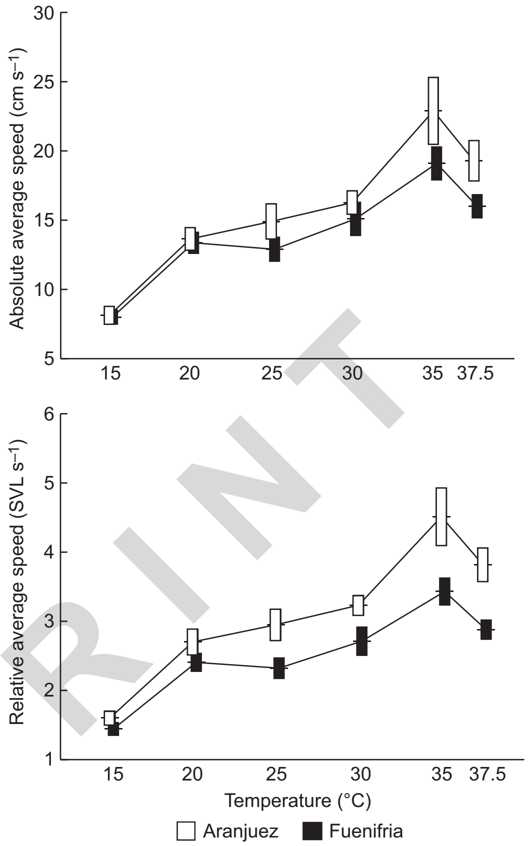

Fig. 4. Mean (± SE) (**a**) absolute average speed (cm s^{-1}) and (**b**) relative average speed (SVL s^{-1}) of *P. hispanica* lizards from the Aranjuez (open boxes) and Fuenfría (black boxes) populations at different temperatures.

Discussion

Here, we showed that two populations of Iberian wall lizards which inhabit areas differing in temperatures differ with respect to body size and coloration as well as some aspects of their thermal biologies. Lizards from the cooler region (northern population Fuenfría) were larger and darker, had slower heating and cooling rates, and attained lower field body temperatures than lizards from the warmer region (southern population Aranjuez). Lizards of the two populations also selected different temperatures in a thermal gradient. Surprisingly, lizards from the colder locality (Fuenfría) selected higher temperatures. Finally, tests of speed indicated that lizards had similar temperature-dependent locomotor performance curves, but that lizards from Aranjuez were faster in relative terms. These results suggested that environmental differences between populations may have contributed to morphological differentiation which, in addition to changes in some aspects of thermal biology, might improve thermoregulation in the climatic conditions of each population.

Morphologically, lizards from the northern population (Fuenfría) are larger, heavier, and have darker dorsal colorations than lizards from the southern population (Aranjuez). Variations in body size of many animals, particularly vertebrates, are often explained by phenotypic plasticity or local adaptation to different climatic conditions. Thus, many species show trends in body size that conform to Bergmann's rule, individuals from colder environments being larger than those from warmer areas (e.g., Yom-Tov & Nix 1986, Olalla-Tárraga *et al*. 2006, Leaché *et al*. 2010). Individuals with large bodies or dark skin have low thermal inertia (i.e., slower cooling rates) and are able to maintain body temperatures for longer (Stevenson 1985, Carrascal *et al*. 1992, Heinrich 1996, Bittner *et al*. 2002, Clusella-Trullas *et al*. 2007, 2009). Therefore, large body size and dark skin may be adaptations to increase efficiency of thermoregulation in the northern population, where ambient temperatures are relatively low, in contrast to the southern population where temperatures are warmer and lizards are smaller and brighter.

Environmental conditions most probably explain why the lizards from the northern population (Fuenfría), attained lower field body temperatures than the lizards from the southern population (Aranjuez) (Díaz 1997, Castilla *et al*. 1999). In view of that, the Fuenfría lizards should also have selected lower temperatures in a thermal gradient than the Aranjuez lizards, but our experiments proved the opposite. This contradictory result might be explained by the fact that the lizards from the northern population, living in an unpredictable mountain environment, try use every opportunity to attain higher body temperatures whenever possible (as in the thermal gradient) (Van Damme *et al*. 1989, Carrascal *et al*. 1992, Díaz 1997). The slower cooling rates of the larger northern lizards would help to maintain their body temperature close to the optimum for longer even if ambient temperatures later decreased (as it is likely to occur in the mountains). On the other hand, the southern lizards can heat faster and have more opportunities to attain high body temperatures throughout the day, in the more predictable thermal conditions of the warm southern plains. Thus, the southern lizards may not need to reach as high body temperatures as they could to attain and maintain their optimal temperatures for longer with low thermoregulatory costs. Also, in this population faster cooling rates would allow lizards to cool effectively in case of overheating, which is more likely to be a problem in the hot southern environmental conditions.

In most lacertids, preferred body temperature appears to be evolutionarily conservative and responding slowly to directional selection; moreover, usually there are no interpopulational differences within the same species (Patterson & Davies 1978, Van Damme *et al*. 1989, 1990, Gvozdík & Castilla 2001, Carretero *et al*. 2005, but see Scheers & Van Damme 2002). However, other studies indicated that there is variation in preferred body temperature between forms or types within the *P. hispanica* species complex, (Carretero *et al*. 2006, Veríssimo & Carretero 2009). Carretero *et al*. (2006) showed that the NE form of *P. hispanica* attained lower preferred body temperatures than other not directly related Iberian *Podarcis* species, which may indicate evolutionary changes. In fact, these NE populations have recently been considered to belong to a different species (*P. liolepis*) (Renoult *et al*. 2010). Also, in *P. vaucheri* from S Iberia and N Africa, which has recently been considered a species, there are differences in T_{sel} as compared with other "forms" or species of the *P. hispanica* species complex (Veríssimo & Carretero 2009). Similarly, the differences between our study populations might suggest that these two *P. hispanica* morphotypes or lineages have experienced strong divergent selective pressure (*see* Pinho *et al*. 2007, Carretero 2008) that modified this apparently conservative trait.

Our results also confirmed that as in many lizards (Huey 1981, Bauwens *et al*. 1995), fleeing speed is affected by temperature. In our case, in both populations fleeing speed increased with temperature reaching maximum at the body temperature of 35 °C, and then decreased again at higher body temperatures. In relative terms, the lizards from Aranjuez were faster than those from Fuenfría. Low vegetation cover and higher diversity of potential predators in the area inhabited the southern population (pers. obs.) may might have selected for higher fleeing speeds (Bauwens *et al*. 1995, Melville & Swain 2000). Morphological variation in relation to habitat characteristics is known to occur in several lizard groups (e.g., Losos 1990, Melville & Swain 2000, Herrel *et al*. 2002, Elstrott & Irschick 2004). For example, a study showed intraspecific variation between habitat types (from saxicolous to ground-dwelling) in traits related to locomotion of *P. bocagei* lizards (Kaliontzopoulou *et al*. 2010). This suggests that locomotion adaptations to can arise in a very short evolutionary time in *Podarcis* lizards.

The relationship between body temperature and fleeing speed was similar in both populations, even though we could expect that as a result of environmental selection maximum fleeing speed would be reached at body temperatures that lizards from the two populations are able to attain in their own enviromnents (Huey & Bennet 1987, Bauwens *et al*. 1995, Angilletta 2009). However, a low amount of genetic divergence between these two populations might not have allowed for a more finely-tuned relationship between local temperature and locomotor performance. Alternatively, it is likely that differences in the temperature–fleeing-speed relationship were so small that our study could not detect them.

In summary, we found evidence of differences in some aspects of thermal biology between two morphologically distinct populations of Iberian wall lizards. Adaptations to the environmental condition encountered by each population could explain or be the result of the morphological and genetic divergence between these populations. Further studies should examine in more detail the degree of adjustment of these differences in thermal biology to the physiological performance optima. Finally, we should also study the actual thermoregulatory behavior of lizards in the field (i.e., activity levels, basking behavior, microhabitat selection, etc) to fully understand the role of climatic variations and thermal adaptations in genetic divergence and speciation process within this *P. hispanica* species complex.

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