

RESEARCH ARTICLE

Effect of body mass and melanism on heat balance in *Liolaemus* lizards of the *goetschi* clade

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ABSTRACT

The body temperature of ectotherms depends on the environmental temperatures and behavioral adjustments, but morphology may also have an effect. For example, in colder environments, animals tend to be larger and to show higher thermal inertia, as proposed by Bergmann's rule and the heat balance hypothesis (HBH). Additionally, dark coloration increases solar radiation absorption and should accelerate heat gain (thermal melanism hypothesis, TMH). We tested Bergmann's rule, the HBH and the TMH within the *Liolaemus goetschi* lizard clade, which shows variability in body size and melanistic coloration. We measured heating and cooling rates of live and euthanized animals, and tested how morphology and color affect these rates. Live organisms show less variable and faster heating rates compared with cooling rates, suggesting behavioral and/or physiological adjustments. Our results support Bergmann's rule and the HBH, as larger species show slower heating and cooling rates. However, we did not find a clear pattern to support the TMH. The influence of dorsal melanism on heating by radiation was masked by the body size effect in live animals, and results from euthanized individuals also showed no clear effects of melanism on heating rates. Comparison among three groups of live individuals with different degrees of melanism did not clarify the influence of melanism on heating rates. However, when euthanized animals from the same three groups were compared, we observed that darker euthanized animals actually heat faster than lighter ones, favoring the TMH. Although unresolved aspects remain, body size and coloration influenced heat exchange, suggesting complex thermoregulatory strategies in these lizards, probably regulated through physiology and behavior, which may allow these small lizards to inhabit harsh weather environments.

KEY WORDS: Bergmann's rule, Cold climates, Heat balance hypothesis, Heating and cooling rates, Thermal inertia, Thermal melanism hypothesis

INTRODUCTION

Temperature is vital for numerous biological processes in ectotherms (Angilletta et al., 2006), playing a significant role in digestion, muscle performance and development (Huey, 1982; Angilletta, 2001; Ragland and Kingsolver, 2008). Therefore, a deviation from an animal's optimal body temperature may reduce fitness (Angilletta et al., 2002). Additionally, the body temperature

of ectotherms depends both on the magnitude of the environmental thermal variability and on the organism's ability to control heat exchange (Carrascal et al., 1992; Belliure and Carrascal, 2002), as reptiles regulate heat exchange mainly through behavioral adjustments (Huey, 1982; Stevenson, 1985; Bartholomew, 1987; Diaz et al., 1996). Movements between sun and shade, activity periods, and postural changes help reptiles modify heat exchange rates by altering the solar radiation absorbed (Huey and Pianka, 1977; Bauwens et al., 1996), as well as infrared radiation by conduction or convection of heat in the surrounding environment (Diaz, 1991; Belliure et al., 1996). Physiological adjustments are also possible through modifications of heart rate or blood flow circulation to appendages. These mechanisms have been observed in reptiles over 20 g with significant effects (Turner and Tracy, 1983; Dzialowski and O'Connor, 1999; Grigg and Seebacher, 1999; Seebacher, 2000; Seebacher and Grigg, 2001), whereas in smaller ectothermic animals the physiological influence is less significant.

Besides behavior, morphological traits also may be important for thermoregulation. For example, body size influences heating and cooling rates, final equilibrium temperatures and thermal inertia in reptiles (Porter and Tracy, 1983; Stevenson, 1985; Carothers et al., 1997; Heatwole and Taylor, 1987; Carrascal et al., 1992; Labra et al., 2009). The lower surface-to-volume ratio of larger animals implies higher heat conservation capacity, or thermal inertia, as Bergmann (1847) postulated. Therefore, larger animals are expected to occur in colder environments than smaller animals. This rationale is straightforward for endotherms, such as mammals (Ashton et al., 2000). However, thermoregulatory capacity may play an important role in the way ectotherms maintain gained heat. Two extreme thermoregulatory strategies are recognized in ectotherms. Whereas thermoregulating animals actively control their body temperatures, thermoconforming animals show temperatures that encompass the environmental temperature. Thus, for thermoregulating ectotherms, a higher heat conservation capacity associated with a larger body size may be advantageous in cold climates. This statement coincides with Bergmann's rule and the underlying heat conservation mechanism proposed for its application in ectotherms (Bergmann, 1847; Gaston and Blackburn, 2000). In contrast, thermoconforming animals should be favored in colder climates by small body sizes because of a higher surface-to-volume ratio, enabling shorter heating times. These two pathways correspond to the so-called heat balance hypothesis (HBH) (Olalla-Tárraga and Rodríguez, 2007). For thermoregulating ectotherms, however, a larger body size also implies longer heating times, and additional costs for basking (Cruz et al., 2005). Therefore, a compensatory mechanism to overcome this disadvantage may be necessary.

Melanism, the dark coloration of organisms, may act as such a compensatory mechanism, given that skin reflectance has a direct effect on the amount of solar radiation absorbed by an organism (Porter and Gates, 1969; Belliure et al., 1996; Angilletta et al., 2006;

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List of symbols and abbreviations

AIC _c	Akaike's information criterion corrected for small sample size
BM	body mass (log ₁₀ transformed)
HBH	heat balance hypothesis
MDOR	dorsal melanism
MTOT	total melanism
MVEN	ventral melanism
NG	net heat gain
PGLS	phylogenetic generalized least squares
<i>t</i>	time (s)
<i>T_a</i>	ambient temperature
<i>T_i</i>	environmental temperature at the beginning of the experiment
TMH	thermal melanism hypothesis
<i>W_i</i>	Akaike weight
<i>W_{i-BM}</i>	Akaike weight excluding the model with body mass as a predictor variable
λ	Pagel's phylogenetic signal
τ	thermal time constant
τ_C	thermal time constant of the cooling rate
τ_{Ce}	thermal time constant of the cooling rate of euthanized animals
τ_{HC}	thermal time constant of the conduction heating rate
τ_{He}	thermal time constant of the radiation heating rate of euthanized animals
τ_{HR}	thermal time constant of the radiation heating rate

Clusella-Trullas et al., 2009). The thermal melanism hypothesis (TMH) proposes that individuals with low reflectance (dark coloration) will gain heat faster than those with high reflectance (light coloration) at the same body size (Norris, 1967; Watt, 1968; Kettlewell, 1973; Gates, 1980), favoring darker organisms in cold environments. The effect of melanistic coloration on heating rates was verified for vipers (Bittner et al., 2002) and *Cordylus* lizards (Clusella-Trullas et al., 2009). However, the importance of heat balance as a selection force contributing to geographic variation in body size and melanism in lizards is not well understood.

Lizards of the *Liolaemus goetschi* Müller & Hellmich 1938 species group follow Bergmann's pattern, with larger body sizes observed at higher latitudes, in association to thermal environmental variables (Moreno Azócar, 2013; Moreno Azócar et al., 2015), suggesting that climate may mold body size distribution within these lizards. Additionally, there is a strong positive relationship between body size and melanistic coloration, with larger, darker lizards inhabiting lower temperature environments (Moreno Azócar et al., 2015). These trends suggest that melanism as well as body size may be related to the speed of heat gain and necessitate empirical validation.

Lizards in the *Liolaemus goetschi* species group are distributed across a 2400 km north–south range (Fig. 1), within which most of the species occur in the harsh Patagonian environments of Argentina. These lizards are small to medium sized (60–105 mm snout–vent length, 4.3–24.4 g) heliotherms, and are efficient thermoregulators (Moreno Azócar et al., 2013). These characteristics make them a good study subject to test the HBH (Olalla-Tárraga and Rodríguez, 2007), the heat conservation mechanism (Bergmann, 1847; Gaston and Blackburn, 2000) and the TMH (Norris, 1967; Clusella-Trullas et al., 2009).

Based on the above information, we aim to test whether HBH is applicable to these lizards, whether thermal inertia can explain Bergmann's pattern in ectotherms, and how body size and melanism affect heating rates, as the TMH proposes. To do this, we expect to address two questions: (1) is body size related to heating and cooling rates in this group of lizards; and (2) does melanism affect heat gain,

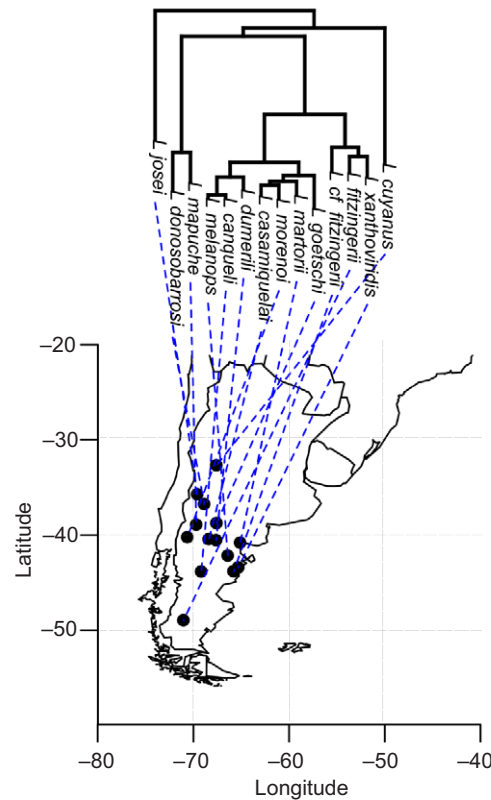


Fig. 1. Geographic localization of the sampled *Liolaemus goetschi* lizard species and their phylogenetic relatedness.

compensating for the body size effect? Because physiology and behavior may influence heat gain, we decided to control these effects by comparing heat exchange using live and euthanized animals. We expect that body size will slow down heat exchange rates whereas melanism will accelerate heating rates. Therefore, larger and darker lizards should present slower cooling rates, but heating rates similar to those of smaller, lighter colored animals.

MATERIALS AND METHODS

Field work

Field work was carried out in February and December 2009. Adult lizards belonging to 14 species of the *Liolaemus goetschi* group (259 individuals in total) were caught by noose or by hand at different locations (Fig. 1). Captured lizards were transported to the laboratory in cloth bags, separated by species, and following ethical animal care proceedings. All captures were authorized by the corresponding Argentinean Provincial fauna offices or by National Parks of Argentina.

Animal housing and care

During resting periods, lizards were kept in cloth bags with adequate humidity at 20°C room temperature. Every 3 days lizards were fed with live crickets in a 1.2×0.6×0.4 m (length×width×height) terrarium with a 20–48°C thermal gradient. The terrarium was subdivided into five 0.12-m-wide lanes where lizards were kept alone until they actually fed. Water was also sprayed and offered *ad libitum* for hydration.

As stress may alter physiology, behavior and hence our results, animals showing possible stress signals (e.g. sudden changes of temperatures, escape behavior, attempts to dig, panting for too long at low temperatures) were not included in the present analyses. We are

aware that long periods in cloth bags may affect the behavior of lizards, but we gave them the opportunity to feed every 3 days in open space (1.2 m lanes with an 18–42°C thermal gradient). Additionally, the temperature and illumination into the cloth bags may help to save energy (and consequently to reduce stress) as a consequence of lower activity.

Laboratory work

Body mass and melanism

Body mass as a measure of body size was measured in the laboratory with a balance (Scout-Pro 200 g, Ohaus, GA, USA; accuracy 0.01 g). To estimate the melanism proportion of the specimens, we took pictures of the dorsal and ventral surfaces of every lizard, under standardized conditions and camera settings, and analyzed them using Photoshop CS3 extended (Adobe Systems) to determine total body surface and melanistic surface (for details, see Moreno Azócar et al., 2015). Ventral melanism was measured because it could affect heating rates, due to lizards absorbing light via reflection from the ground, as these lizards use an erect basking position. Total, dorsal and ventral melanistic surface were coded as a proportion of melanistic surface over the total surface (total, dorsal or ventral surface), to standardize the variables and make them comparable between species (Alho et al., 2010).

Experimental temperature determinations

Heating rate measurements

To measure lizard heating rates, we used two experimental designs considering the two main heat sources used by lizards in nature: a ‘radiation’ experiment (HR), where the animal temperature is obtained by dorsal solar radiation absorption; and a ‘conduction’ experiment (HC), where the main heat source is the substrate and temperature is obtained by conduction. For the radiation experiment, lizards were placed into a 10 liter white opaque plastic bucket (20 cm diameter, 30 cm high) with a 2 cm layer of sand on the bottom, and an incandescent daylight bulb (Philips, 100 W) suspended 45 cm above the substrate. Although this artificial lighting provides less radiation in the infrared and ultraviolet wavelengths compared with natural sunlight, it has a high color temperature (6500 K), reflecting a visual spectrum similar to a midday natural light, and providing heat at the same time. Nevertheless, body temperatures did rise markedly during the experiments. Although different to an experiment conducted under natural conditions, this setting allowed us to standardize the measurements, making them comparable between species. On the contrary, a natural conditions experiment would introduce more noise into the data (Bittner et al., 2002). Body temperature of individuals at the beginning of both experiments was $15 \pm 0.2^\circ\text{C}$ (mean \pm s.e.m.), and air temperature inside the bucket was 35°C , according to air temperatures (1 cm above the ground) recorded in the field. For every measurement, the lamp was turned on 1 min before the beginning of the trial. The individual was placed into the bucket to allow heat gain, and once the lizard reached a body temperature of 35°C the bulb was turned off. We measured body temperature with a type K thermocouple attached to the lizard’s lateral body surface (Moreno Azócar et al., 2013), plugged into a temperature data collector (Omega HH147U, Omega Engineering, Stamford, CT, USA). Body temperature was registered every 15 s. Substrate and air temperatures were monitored every 2 min using a similar thermocouple. To set up the conduction experiment we used a commercial heating stone (15 cm diameter) for reptiles in the bottom of the bucket covered by 2 cm of sand (the stone was set to provide a constant sand temperature of 35°C by the incorporation of

a thermostat). We provided light with low heat emission (a fluorescent daylight bulb with luminosity equivalent to a 100 W daylight incandescent bulb) following the same protocol as above. After conduction heating rate trials we allowed the lizards to recover in a different terrarium with a thermal gradient, and they were fed and hydrated *ad libitum*. When possible, we used different specimens for radiation and conduction experiments, but a small number of individuals of some species were used in both trials [e.g. *L. cuyanus* ($N=6$), *L. donosobarrosi* ($N=2$), *L. goetschi* ($N=7$), *L. melanops* ($N=6$) and *L. morenoi* ($N=4$)]; nonetheless, the lizards had a resting period of at least 2 days between trials.

Cooling rate measurements

To obtain cooling rates we placed the lizards into a 10 liter plastic bucket (20 cm diameter) immediately after finishing radiation heating rate measurements, within a walk-in environmental chamber with controlled air temperature ($15 \pm 0.5^\circ\text{C}$). The bucket was at environmental temperature. Light was provided by a low heat emission, fluorescent bulb (luminosity equivalent to 100 W daylight incandescent bulb). We measured lizards’ body temperature change from 35 to 15°C following the same procedure used for heating rates. We allowed lizards to recover their body temperature in a thermal gradient after measurements, as above.

After all measurements were finished, lizards were euthanized by lethal injection (under the ethics convention of CONICET ethics bureau), fixed in 5% formaldehyde and later transferred to 70% ethanol for preservation. We then measured heating and cooling rates of the euthanized animals (previously drained by a small cut in the belly and dried externally by rubbing them with paper towels) to account for the heat exchange rates of body size and color in the absence of physiology and behavior adjustments. To do this we followed the exact protocols described above for live animals, but heating rates were measured from 15 to 30°C only for the radiation experiment, and cooling rates were measured from 30 to 18°C , because of the longer time it took in many cases to achieve the equilibrium temperatures. After we finished the experiments, euthanized animals were deposited in the herpetological collection of the Instituto de Herpetología (FML, Tucumán, Argentina).

Phylogenetic framework

We used the phylogenetic tree constructed by using aligned DNA sequences (1726 base pairs) and *a priori* partitioned maximum likelihood (ML) analysis for the 14 species of the *Liolaemus goetschi* group from Moreno Azócar et al. (2015) (Fig. 2).

Statistical analyses

We first tested for sexual differences within species in all the parameters studied, using a two-way ANOVA with species and sex as factors. After finding no differences (see Table S1), we calculated the mean values of all variables for each species. When necessary, we transformed the data to approximate a normal distribution. Body mass (BM) was \log_{10} transformed for all the analyses performed here. For total, ventral and dorsal melanism proportions (total melanism = total melanistic surface \times 100 / total body surface; ventral melanism = ventral melanistic surface \times 100 / ventral body surface; dorsal melanism = dorsal melanistic surface \times 100 / dorsal body surface) we used the arcsine of the square root transformation (MTOT, MVEN and MDOR, respectively).

We measured heating and cooling rates as thermal time constants (τ ; Bell, 1980; Cossins and Bowler, 1987), following

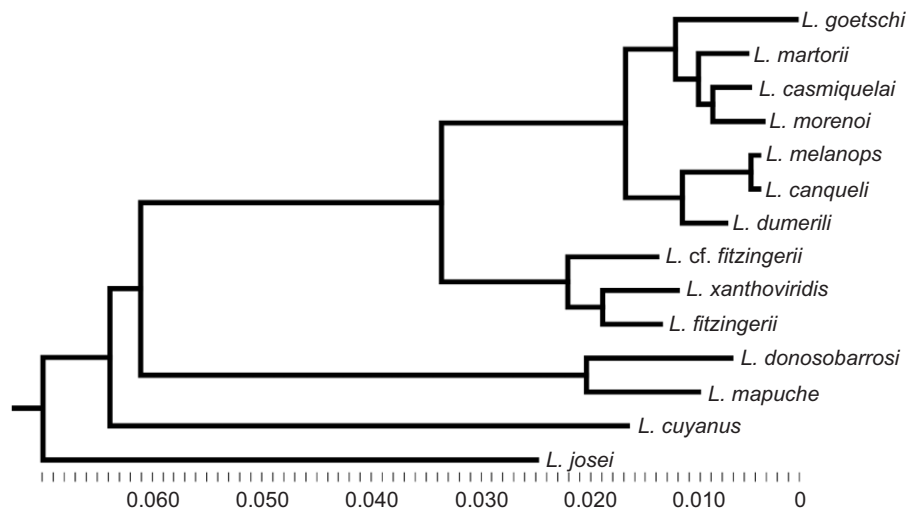


Fig. 2. Phylogenetic relationships of the *Liolaemus goetschi* species group according to Moreno Azócar et al. (2015). Scale indicates evolutionary time.

Labra et al. (2009). We estimated the constants for every species and rate as $\tau = -0.4343/b$, where b is the slope of $\ln(T_i - T_a)$ against time, T_i is the body temperature of the experimental animal recorded at different time points and T_a is ambient (air 1 cm above the substrate) temperature, which was held constant at 35°C for heating and 15°C for cooling. τ values vary from 0 to infinite; the smaller the value, the faster the heat exchange. We obtained three different thermal time constants for live individuals – radiation heating (τ_{HR}), conduction heating (τ_{HC}) and cooling (τ_C) rates – while for euthanized individuals we obtained constants for radiation heating (τ_{He}) and cooling (τ_{Ce}). We also estimated net heat gain (NG) species average values following Zamora-Camacho et al. (2014). However, as heating and cooling curves of our measurements were not linear, we used the slope as measured above $[\ln(T_i - T_a)/t]$, which differs from that used by Zamora-Camacho et al. (which was $T_{final} - T_{initial}/t$, where T_{final} and $T_{initial}$ are the final and initial temperatures, respectively, and t is the time elapsed during the experiment). For every variable we averaged individual data per species to obtain a unique value used in comparative analyses (Table S1).

The influence of size and color, as well as geographic and climatic variables, was analyzed with phylogenetic generalized linear models (PGLS) in order to include the species' phylogenetic relationships (Harvey and Pagel, 1991; Martins, 1996), for live and euthanized species mean values separately. PGLS estimates Pagel's phylogenetic signal (λ) from the residual errors of the regression parameters simultaneously. In most cases, this procedure performs equivalent either to phylogenetic or to non-phylogenetic procedures depending on the λ value obtained (Revell, 2010), and has the effect of simplifying statistical procedures and reducing Type I error rates. As collinearity was observed among melanism variables, we separated them to avoid this. We ran PGLS models using τ_{HR} , τ_{HC} , τ_C or NG as dependent variables, and BM, MTOT, MVEN, MDOR and the interaction of every melanism variable with body size as predictor variables. Because of the strong influence of body size on heat exchange rates, we estimated the strength of the models for the whole set (including BM), and only for melanism variables (excluding the model with BM as a predictor, but leaving those with the interaction of BM and melanism). We also ran an ANCOVA comparing heating rates among light (<10% melanic), medium (10–25% melanic) and dark individuals (>25% melanic, arbitrarily chosen), with species pooled together.

To ensure the regression model provided the best fit among the candidate models (Angilletta et al., 2006), we used Akaike's information criterion (AIC). Akaike weights (W_i) were used as a measure of strength of evidence for each model, indicating the probability that a given model is the best among a series of candidate models (Burnham and Anderson, 2004). All analyses were performed and plots were generated using the freely available software R (R Core Team, 2014), with the packages ape (Paradis et al., 2004), caper (Orme et al., 2013), geiger (Harmon et al., 2008), ggpolt2 (Wickham, 2009), gtools (Warnes et al., 2014), nlme (Pinheiro et al., 2015), phytools (Revell, 2012) and picante (Kembel et al., 2010).

RESULTS

Heating and cooling rates, and net heat gain

Body mass varied roughly fivefold across species of the *L. goetschi* group, from 4.29 ± 0.89 g (mean \pm s.d.) in *L. donosobarrosi* to 24.40 ± 5.42 g in *L. casmiquelai*. These species also show variation in the degree of melanism (for details, see Moreno Azócar et al., 2015), where *L. donosobarrosi* is the lighter species, with almost no melanic areas (MTOT 0.04%), and *L. canqueli* is the darkest (MTOT 44.79%).

After finding no intersexual differences within species on heating and cooling rates in any of the studied rates (two-way ANOVA, $P > 0.719$ in all cases; see Table S1), we pooled the sexes together for comparative analyses. Live lizards of the *L. goetschi* group showed low variation among their heating rates when the source was radiation, with thermal time constant values between 2.15 and 6.17 (Table S2). In contrast, conduction heating thermal time constants were much higher (that is, slower rates) and also variable, with τ values between 9.26 and 17.20 (Table S2). Cooling rates were the most variable, with τ values between 2.42 and 15.80 (Table S2). For euthanized lizards, heating τ values varied between 4.37 and 11.01, while registered cooling τ values were between 3.5 and 8.5 (Table S3). An opposite trend is observed when comparing live versus euthanized animals, with the live organisms showing faster heating than cooling rates, and the euthanized group showing almost no differences among heating and cooling τ values (Fig. 3). In addition, while heating τ values were less variable in live animals compared with cooling τ values, in euthanized animals both thermal time constants showed similar ranges (Fig. 3). Values of net heat gain varied between -0.08 and 0.17 for all *L. goetschi* species (Table S2).

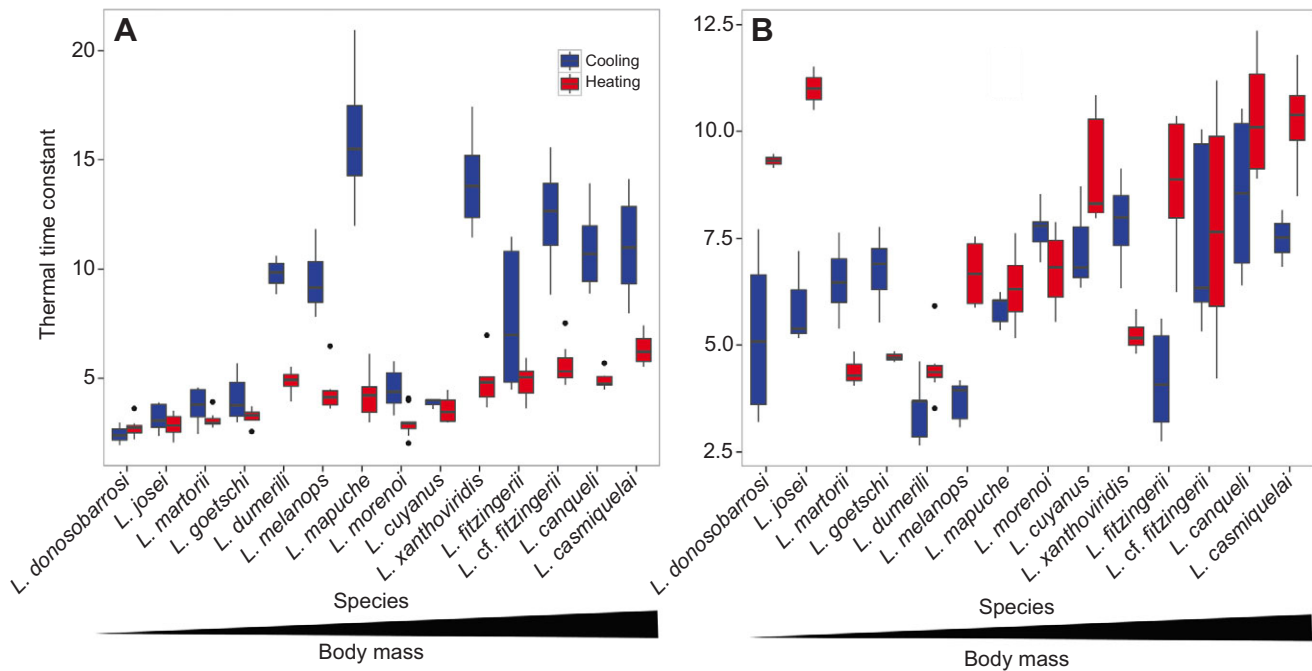


Fig. 3. Thermal time constants of heating and cooling rates of lizards of the *Liolaemus goetschi* group. (A) Living lizards; (B) euthanized lizards. Body mass increases from left to right. Heating rates of live animals are faster (smaller thermal time constants) than cooling rates, and much more homogeneous than heating rates of euthanized lizards, under the same conditions.

Influence of body size and melanism on heating and cooling rates

PGLS analyses testing the influence of morphology (body size and melanism degree) on heating and cooling rates showed a clear influence of body mass on heat exchange thermal time constants, with larger species showing slower rates (higher τ values) for the three rates measured (Table 1, Fig. 3). The model including BM as a predictor variable had the best fit in all cases (Table 1), revealing a strong effect of body mass on τ values. The better fit of these models in the analyses for heating τ of euthanized animals provides additional support for the role of body mass in thermoregulation (Table 2), although in all analyses Pagel's λ was equal to zero, meaning that there was no phylogenetic signal. When we plot an ancestral state reconstruction, we can see differences along the tree in body size as well as heating and cooling rates (Fig. 4).

PGLS analyses did not support the TMH predictions. Radiation heating τ was not influenced by dorsal melanism (Table 1). The relevance of melanism variables may be masked by the influence of body mass (Fig. 5), but we were unable to totally remove this effect. Conduction heating/morphology models showed low r^2 and W_i values (Table 1) in all cases, while cooling τ were only influenced by body mass, both for living and euthanized rates (Tables 1, 2). Different trends in heating τ were observed for the euthanized lizards. The model with the better fit according to its W_i was that including exclusively ventral melanism as predictor variable, even though all models – with the exception of that with MDOR – showed high regression coefficient values. Cooling τ showed no effect of body mass or melanism (Table 2). Remarkably, while PGLS models of live animals for either heating or cooling rates showed no phylogenetic signal, all models of euthanized lizards for heating rates showed high values of Pagel's λ , suggesting that related species are more similar than expected by chance.

The ANCOVA testing for differences in heating rates between light, intermediate and dark lizards (log body mass as a covariate)

showed significant differences among groups for euthanized animals [model, $F=13.306$, $P<0.001$; BM (covariate), $F=39.538$, $P<0.001$; melanism (factor), $F=4.056$, $P=0.025$]. Darker lizards showed the fastest heating rates, while intermediate animals showed the slowest rates. In contrast, the same analysis performed for live animals did not show an effect of melanism group, even when the model was significant [model, $F=3.794$, $P=0.015$; BM (covariate), $F=10.338$, $P=0.002$; melanism (factor), $F=0.053$, $P=0.9448$].

Net heat gain variation

None of the studied relationships using net heat gain as dependent variables show trends of variation in relation with morphology (Table 1).

DISCUSSION

We found that live lizards gain heat faster than they cool, while euthanized animals show the reverse trend (they cool faster than they heat), as observed previously in different species (Bartholomew and Tucker 1963), indicating a physiological and/or behavioral effect on heat absorption (Huey et al., 2003). As previous studies have noted, the influence of thermal inertia (the resistance of a body to changing its temperature) is higher on cooling than on heating rates (Smith, 1976; Claussen and Art, 1981; Carothers et al., 1997; Zamora-Camacho et al., 2014) for live animals. This difference can be explained by behavioral or physiological adjustments to increase heat gain (Lillywhite, 1980; Blouin-Demers and Weatherhead, 2001) that buffer the effect of body size (thermal inertia), commonly observed in lizards (Porter and Tracy, 1983; Carrascal et al., 1992; Carothers et al., 1997; Heatwole and Taylor, 1987; Labra et al., 2009). Behavioral adjustments in the experimental conditions used here may be due to postural changes, a behavior not recorded in the present study that needs further attention. Additionally, modifications of heart rate (Turner, 1987; Seebacher, 2000) and blood flow to the appendages

Table 1. Phylogenetic generalized least squares models evaluating the influence of body size and melanism on heating and cooling rates in the *Liolaemus goetschi* group

Model	λ	r^2	AIC _c	W_i	W_{i-BM}	Intercept	BM	MTOT	MDOR	MVEN
$\tau_{HR} \sim MVEN$	0.000	0.386	44.466	0.196	0.326	3.019**				4.063*
$\tau_{HR} \sim MDOR$	0.000	-0.002	51.307	0.006	0.011	3.391**			1.819	
$\tau_{HR} \sim MTOT$	0.000	0.291	46.477	0.072	0.119	2.879**		3.975*		
$\tau_{HR} \sim BM$	0.000	0.444	43.055	0.398		-0.734	1.914**			
$\tau_{HR} \sim BM+MVEN$	0.000	0.453	44.936	0.155	0.258	0.382	1.277			1.988
$\tau_{HR} \sim BM+MDOR$	0.000	0.405	46.098	0.087	0.144	-0.925	2.076*		-0.7578	
$\tau_{HR} \sim BM+MTOT$	0.000	0.405	46.119	0.086	0.143	-0.264	1.618	0.970		
$\tau_C \sim MVEN$	0.001	0.384	79.503	0.277	0.385	5.927**				14.153*
$\tau_C \sim MDOR$	0.001	0.033	85.813	0.012	0.016	6.899**			7.570	
$\tau_C \sim MTOT$	0.001	0.333	80.610	0.159	0.221	5.244**		14.640*		
$\tau_C \sim BM$	0.001	0.385	79.486	0.280		-6.240	6.289*			
$\tau_C \sim BM+MVEN$	0.001	0.411	80.966	0.133	0.185	-1.632	3.662			8.206
$\tau_C \sim BM+MDOR$	0.001	0.329	82.791	0.054	0.074	-6.322	6.359*		-0.325	
$\tau_C \sim BM+MTOT$	0.001	0.371	81.872	0.085	0.118	-2.927	4.207	6.826		
$\tau_{HC} \sim MVEN$	0.000	0.220	68.054	0.194	0.212	12.088**				6.732*
$\tau_{HC} \sim MDOR$	0.000	0.271	67.110	0.311	0.340	11.462**			7.801*	
$\tau_{HC} \sim MTOT$	0.000	0.246	67.581	0.246	0.268	11.585**		7.684*		
$\tau_{HC} \sim BM$	0.000	0.120	69.741	0.084		7.608*	2.448			
$\tau_{HC} \sim BM+MVEN$	0.000	0.155	71.255	0.039	0.043	10.834*	0.608			5.745
$\tau_{HC} \sim BM+MDOR$	0.000	0.236	69.860	0.079	0.086	9.238*	1.069		6.473	
$\tau_{HC} \sim BM+MTOT$	0.000	0.178	70.874	0.047	0.052	11.124*	0.237	7.243		
NG \sim MVEN	0.000	0.027	-61.761	0.154	0.266**	-0.028				-0.035
NG \sim MDOR	0.000	0.007	-61.474	0.133	0.317*	-0.027			-0.034	
NG \sim MTOT	0.000	0.097	-62.808	0.260	0.148*	-0.024		-0.049		
NG \sim BM	0.000	0.106	-62.954	0.279	0.136	0.014	-0.021			
NG \sim BM+MVEN	0.000	0.026	-59.655	0.054	0.345	0.011	-0.019			-0.004
NG \sim BM+MDOR	0.000	0.034	-59.769	0.057	0.330	0.011	-0.018		-0.012	
NG \sim BM+MTOT	0.000	0.049	-59.987	0.063	0.303	0.001	-0.013	-0.026		

AIC_c, Akaike's information criterion corrected for small sample size; BM, body mass; MTOT, MVEN and MDOR, total, ventral and dorsal melanism, respectively; NG, net heat gain; W_i , Akaike weight; W_{i-BM} , Akaike weight excluding the model with body mass as a predictor variable; λ , Pagel's phylogenetic signal; τ_{HR} and τ_{HC} , radiation and conduction heating rates, respectively; τ_C , cooling rate. Asterisks indicate a significant difference in predictor variable slopes: ** $P < 0.01$, * $P < 0.05$.

(Dzialowski and O'Connor, 1999) may be also involved. However, these latter physiological mechanisms were observed to affect ectothermic animals larger than 100 g (Turner, 1987; Dzialowski and O'Connor, 1999; Seebacher, 2000), and thus the effect on small *Liolaemus* lizards is probably minimal. Nevertheless, it must be considered that despite their small body sizes, *Liolaemus* lizards show particular ecological and physiological characteristics; for example, herbivory (an attribute associated to large body sizes) evolved 65 times faster in this genus of lizards compared with other

reptile clades (Espinoza et al., 2004). This finding prevents us from discarding these physiological mechanisms, which may allow these lizards to shunt blood in an effective way despite their small body size, plus other unknown physiological mechanisms as having an effect on heat absorption.

For ectotherms inhabiting colder environments, longer heat conservation times (slower cooling rates) allow lizards to remain active for longer periods, at the same time increasing time available for other activities than thermoregulation (Clusella-

Table 2. Phylogenetic generalized least squares models evaluating the influence of body size and melanism on heating and cooling rates of euthanized animals in the *Liolaemus goetschi* group

Model	λ	r^2	AIC _c	W_i	W_{i-BM}	Intercept	BM	MTOT	MDOR	MVEN
$\tau_{He} \sim MVEN$	1.000	0.827	61.786	0.410	0.498	8.054**				8.809**
$\tau_{He} \sim MDOR$	0.492	0.258	64.318	0.116	0.141	7.012**			6.608*	
$\tau_{He} \sim MTOT$	1.000	0.790	64.527	0.104	0.127	7.406**		10.371**		
$\tau_{He} \sim BM$	1.000	0.805	63.473	0.177	NA	-0.528	4.028**			
$\tau_{He} \sim BM+MVEN$	1.000	0.820	64.416	0.110	0.134	5.030	1.412			5.875
$\tau_{He} \sim BM+MDOR$	1.000	0.793	66.412	0.041	0.049	1.080	3.085		2.673	
$\tau_{He} \sim BM+MTOT$	1.000	0.794	66.330	0.042	0.051	2.174	2.646	3.693		
$\tau_{Ce} \sim MVEN$	0.000	-0.019	56.867	0.098	0.164	5.955**				1.819
$\tau_{Ce} \sim MDOR$	0.000	-0.024	56.939	0.094	0.158	5.848**			1.869	
$\tau_{Ce} \sim MTOT$	0.000	-0.015	56.811	0.101	0.169	5.824**		2.056		
$\tau_{Ce} \sim BM$	0.000	0.168	54.032	0.404		1.902	1.796			
$\tau_{Ce} \sim BM+MVEN$	0.000	0.142	56.554	0.114	0.192	0.092	2.733			-2.422
$\tau_{Ce} \sim BM+MDOR$	0.000	0.095	57.299	0.079	0.132	1.718	1.919		-0.468	
$\tau_{Ce} \sim BM+MTOT$	0.000	0.138	56.620	0.111	0.185	0.297	2.703	-2.557		

AIC_c, Akaike's information criterion corrected for small sample size; BM, body mass; MTOT, MVEN and MDOR, total, ventral and dorsal melanism, respectively; W_i , Akaike weight; W_{i-BM} , Akaike weight excluding the model with body mass as a predictor variable; λ , Pagel's phylogenetic signal; τ_{He} and τ_{Ce} , radiation heating and cooling rate of euthanized animals. Asterisks indicate a significant difference in predictor variable slopes: ** $P < 0.01$, * $P < 0.05$.

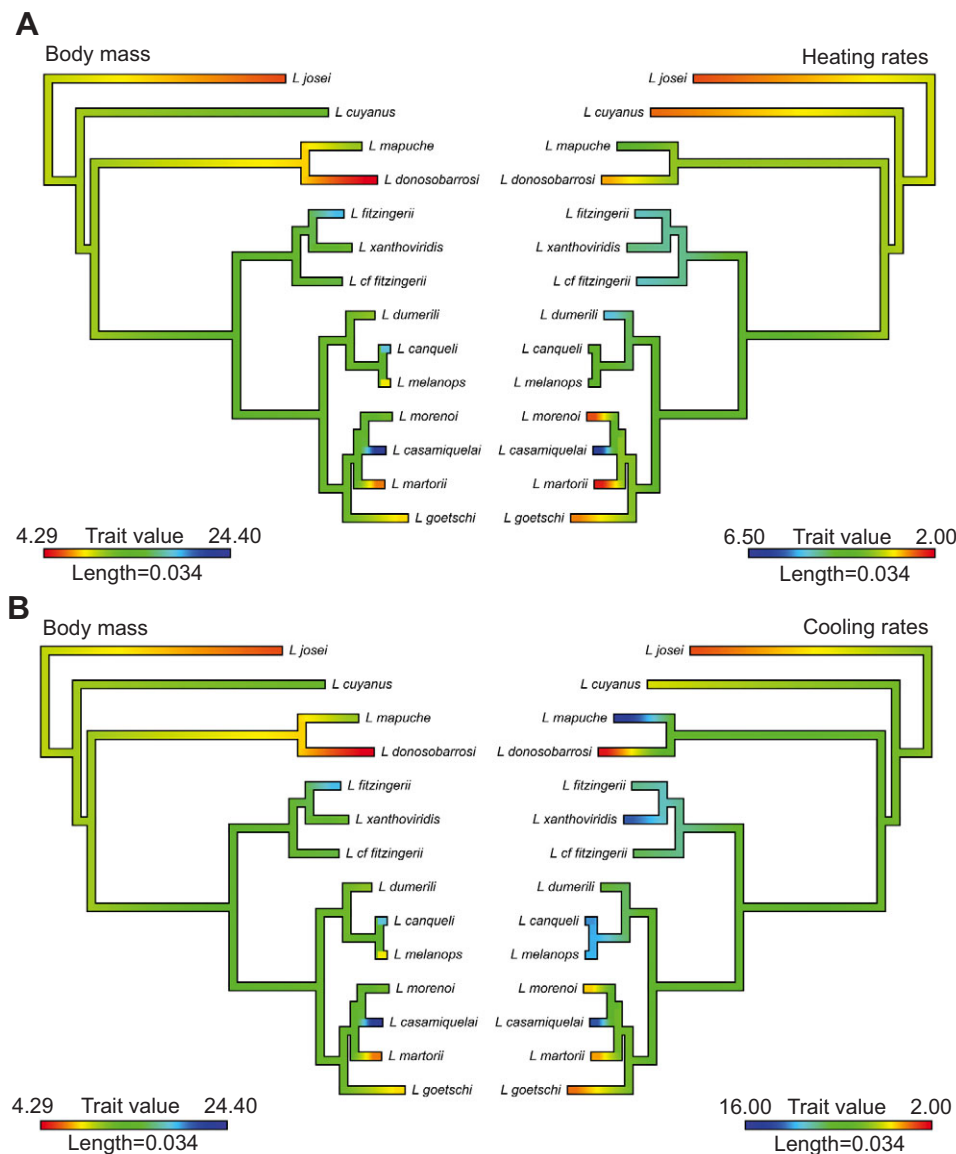


Fig. 4. Ancestral states reconstruction of body mass and heating and cooling rates of live animals of the *Liolaemus goetschi* group. (A) Heating rates; (B) cooling rates. The figure highlights the influence of body mass on heat exchange in these lizards. Redder and yellower colors indicate smaller body masses and faster heating rates.

Trullas et al., 2007); thus, increased growth rates may be favored (Tanaka, 2009). Because the lizards studied here are good thermoregulators (Moreno Azócar et al., 2013), they are able to sustain activity under suboptimal thermal environments such as in Patagonia, as was suggested for other *Liolaemus* species (Fernández et al., 2011). For these lizards, a larger body size represents an advantage for heat conservation as it delays cooling rates, and it has little or no disadvantageous effect on heat gain, supporting the heat balance hypothesis for small thermoregulating animals (Olalla-Tárraga and Rodríguez, 2007). The relationship between body size and cooling rates, with larger lizards showing slower cooling rates, provide evidence for the evolutionary advantages of body size increment at higher latitudes or colder environments. Moreover, the ability of these lizards to overcome the delaying effect of larger body size on heat gain is evident after the comparison between heating rates in living and euthanized animals. Heating rates of living animals are much faster than those of dead lizards, especially at larger body sizes, meaning that lizards accelerate heat gain either through behavioral (postures) or physiological strategies (blood shunt). In the same way, larger live lizards have the ability to delay heat loss, thus showing slower

cooling rates than euthanized animals. As such, Bergmann's rule is consistent here as a result of the role of thermal inertia due to body size (Gaston and Blackburn, 2000) and the thermoregulatory ability of these ectotherms. Also, our findings are in agreement with those of Zamora-Camacho et al. (2014), who studied different populations of *Psammodromus algirus* inhabiting an altitudinal gradient. These authors found that thermal inertia is relevant for cooling rather than heating rates. They also suggested that heating and cooling rates are not always a good way to measure the effect of thermal inertia, and recommend using net heat gain as a trait that better reflects the benefits of thermal inertia. In the present study, we attempted to follow their method, but failed to find any significant trend of net heat gain. The differences between the ways of measuring heating and cooling rates (constants versus net gain or loss) may be related to the scale of the results obtained, being larger in the case of the thermal time constants and therefore more notable. Another possible reason for the differences in the results between the present study and that of Zamora-Camacho et al. (2014) is the type of function for obtaining heating and cooling rates used, as they used a linear increment (or decrement) whereas we used an exponential curve.

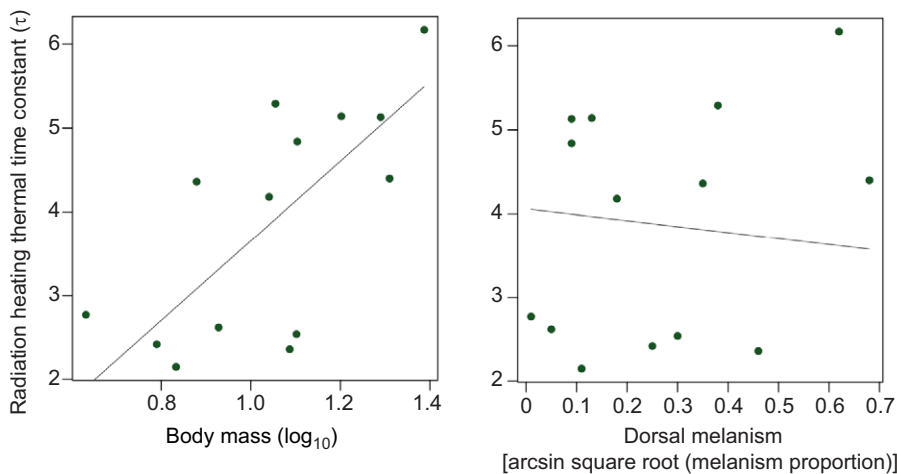


Fig. 5. Partial plots of the phylogenetic generalized least squares model showing the influence of body mass (left) and dorsal melanism (right) on thermal time constants of radiation heating rates. Although the influence of body size is significant, the effect of dorsal melanism was weak and not significant.

To some extent, heating and cooling rates of *L. goetschi* lizards reflect the solar radiation availability in the environments they inhabit, being slower at heating and/or cooling at higher latitudes, in relation to the cloudiness in the area (data not shown, see Moreno Azócar et al., 2015). Such a relationship may be explained by incremental changes in body size with latitude or by constraints of behavioral adjustments to overcome low temperatures or solar radiation. The evolutionary implications of these results show the lack of an adaptive response to climatic variables, phylogenetic constraints, physiological mechanisms such as differential blood flow, or an alternative and needed compensatory strategy, such as behavioral adjustments (Christian et al., 2006).

Body mass partially explains the presence of larger *Liolaemus* species in cool environments. We proposed and explored melanism as a compensatory (or complementary) mechanism that may help these ectotherms achieve temperatures close to the optimum at a faster rate under the disadvantageous thermal conditions of Patagonia. Such reasoning arose from the positive relationship between size and melanism previously found by Moreno Azócar et al. (2015). It is important to note that the effects of body size and melanism co-varied, making it difficult to tease apart the effects of these variables, and both variables may be compensating for each other (thermal inertia due to body mass and heat gain rate acceleration due to melanism).

Surprisingly, we did not observe any influence of dorsal melanism on heating rates, either because it has no real effect, or because body mass influence overwhelms its effect. PGLS models used to analyze the influence of morphology on the heating rates of euthanized lizards did not clarify these relationships. Similar to our findings in live lizards, the most important variable for euthanized animals was body mass. The influence of dorsal melanism was evident, however, when we analyzed differences among euthanized individuals of similar sizes with different coloration, finding that darker lizards gain heat faster than intermediate and light specimens, taking in consideration their body mass. These results prevent us from being conclusive about the effect of melanism on heating rates.

It has to be considered that melanism does not have a unique function. It has been considered an accelerator for heat gain (e.g. Kettlewell, 1973; Kingsolver and Wiernasz, 1991; Clusella-Trullas et al., 2007; Reguera et al., 2014); however, it also is associated with UV radiation protection (Gunn, 1998; Callaghan et al., 2004; Calbó et al., 2005; Reguera et al., 2014), crypsis (Kettlewell, 1973; Endler, 1984; Reguera et al., 2014) and intra-specific communication or sexual selection (Wiernasz, 1989; Fedorka et al., 2013). Additionally, the advantages of melanism may be associated with

local conditions (local adaptation; Castella et al., 2013), as a result of phenotypic plasticity (Alho et al., 2010) or the interaction between predator avoidance and thermoregulation (Lindstedt et al., 2009). Thus, the role of melanism is more complex than expected, or at least not exclusively related to thermal biology (Tanaka, 2009). The complex nature of melanism has been observed previously in ectotherms (Tanaka, 2007, 2009; Janse van Rensburg et al., 2009; Harris et al., 2012; Castella et al., 2013; Roff and Fairbairn, 2013) and some studies have considered melanism to be non-adaptive (e.g. Strugariu and Zamfirescu, 2011).

Therefore, several selective forces may be acting on melanism variation in synchrony. In addition, melanism may have different effects depending on the type of ectothermic organism (vertebrate or invertebrate) and may be limited by body size (Bittner et al., 2002). For example, lizards smaller than 5 g did not show differences in their heating rates between melanic and striped morphs, but larger animals did (Crisp et al., 1979; Bittner et al., 2002). Additionally, Shine and Kearney (2001) proposed that the adaptive advantage of melanism in ectotherms may be restricted to relatively large animals. Other factors to be considered are microclimatic conditions, refuge orientation towards the sun and thermoregulatory behavior, which has been observed to be efficient in this species group (Moreno Azócar et al., 2013).

According to the TMH, skin reflectance affects the quantity of solar radiation absorbed by an organism in a direct way (Norris, 1967; Watt, 1968; Kettlewell, 1973; Gates, 1980; Clusella-Trullas et al., 2009). Therefore, individuals inhabiting cold climates are expected to benefit from having melanic coloration, and effective thermoregulation should result in greater fitness (Clusella-Trullas et al., 2007). However, these statements are mainly supported in arthropods (Pereboom and Biesmeijer, 2003), whereas the studies conducted in ectothermic vertebrates provided conflictive results (supporting TMH: Gibson and Falls, 1979; Bittner et al., 2002; Clusella-Trullas et al., 2009; Castella et al., 2013; contrary to TMH: Forsman, 1995; Tanaka, 2007, 2009). Our results are somewhat mixed too, although the increasing effect of dorsal melanism on heating rates of living animals supports the TMH.

In addition, the effect of melanism on radiation absorption is complex and depends on several aspects, such as behavioral adjustments, configuration of the integument structures (Porter, 1967), and other characteristics of the skin, such as shine and iridescence. Additionally, many lizard species physiologically regulate body color according to environmental thermal conditions, e.g. chameleons, agamas and geckos (Janse van Rensburg et al., 2009), as possibly do *Liolaemus* lizards, but to a smaller extent (they

turn darker at lower environmental temperatures; F.B.C., unpublished data). The skin darkening may increase the rate of heat absorption, because of the higher radiation absorption of dark colors, and making heating less linear than models predict. For example, lizards may turn paler with an increase in body temperature, and at the same time the heating rate may change. Further analyses, including experimental and field data of thermoregulatory behavior, plus comparison of similar sized species with different degrees of melanism, may help clarify whether the thermoregulatory function of melanism has adaptive significance in ectotherms. We acknowledge that the measure of melanism used here (proportion of body surface with black color) may not accurately represent skin reflectance. Therefore, a more accurate measurement of reflectance (e.g. using a reflectometer), analyzing temporal variation of lizard color, would help us to obtain more precise results.

The observed effect of body size on heating rates also may be compensated by behavioral adjustments (shuttling between sun and shade, postural adjustments, etc.; Olalla-Tárraga and Rodríguez, 2007). During our field trips we observed that the larger and southern species were captured under large bushes (*Prosopis*, *Lycium* sp.); these lizards were under the filtered sun where a sun/shadow net makes it difficult to observe them. It is possible that their thermoregulatory ability allows them to exploit a suboptimal thermal environment, avoiding overheating and predator detection at the same time (Forsman, 2011). We also observed that smaller *Liolaemus* lizards are more active during the colder months when adult lizards remain retreated, such as in *L. chacoensis* (Fitzgerald et al., 1999). Thus, local adaptation and use of different microclimate conditions should be considered for species groups with varying degrees of melanic coloration (Alho et al., 2010).

In conclusion, body mass and dorsal melanism affect heat exchange in lizard species of the *Liolaemus goetschi* group. Cooling is delayed by body size, while the effect of melanism increases heat gain, although its effect is masked by body mass. We provide support for the heat balance hypothesis and the thermal melanism hypothesis, although further studies are required.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Field work was done by D.L.M.A., M.F.B., M.G.P., F.B.C. and C.S.A. Experimental work and laboratory duties were carried out by D.L.M.A., M.F.B., M.G.P. and F.B.C. DNA analyses for phylogenetic and systematic data were done by J.A.S. Statistical analyses were performed by D.L.M.A. and F.B.C. All authors contributed to manuscript preparation.

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Supplementary information

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References

- Alho, J. S., Herczeg, G., Söderman, F., Laurila, A., Jönsson, I. and Merilä, J. (2010). Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? *BMC Evol. Biol.* **10**, 317.
- Angilletta, M. J. (2001). Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* **82**, 3044–3056.
- Angilletta, M. J., Hill, T. and Robson, M. A. (2002). Is physiological performance optimized by thermoregulatory behavior? A case study of the eastern fence lizard, *Sceloporus undulatus*. *J. Therm. Biol.* **27**, 199–204.
- Angilletta, M. J., Jr, Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F. and Wilson, R. S. (2006). Coadaptation: a unifying principle in evolutionary thermal biology. *Physiol. Biochem. Zool.* **79**, 282–294.
- Ashton, K. G., Tracy, M. C. and De Queiroz, A. (2000). Is Bergmann's rule valid for mammals? *Am. Nat.* **156**, 390–415.
- Bartholomew, G. A. (1987). Interspecific comparison as a tool for ecological physiologists. In *New Directions in Ecological Physiology* (ed. M. E. Feder, A. F. Bennett, W. W. Burggren and R. B. Huey), pp. 11–37. Cambridge: Cambridge University Press.
- Bartholomew, G. A. and Tucker, V. A. (1963). Control of changes in body temperature, metabolism, and circulation by the agamid lizard, *Amphibolurus barbatus*. *Physiol. Zool.* **36**, 199–218.
- Bauwens, D., Hertz, P. E. and Castilla, A. M. (1996). Thermoregulation in a lacertid lizard: the relative contributions of distinct behavioral mechanisms. *Ecology* **77**, 1818–1830.
- Bell, C. J. (1980). The scaling of the thermal inertia of lizards. *J. Exp. Biol.* **86**, 79–85.
- Belliure, J. and Carrascal, L. M. (2002). Influence of heat transmission mode on heating rates and on the selection of patches for heating in a Mediterranean lizard. *Physiol. Biochem. Zool.* **75**, 369–376.
- Belliure, J., Carrascal, L. M. and Díaz, J. A. (1996). Covariation of thermal biology and foraging mode in two Mediterranean lacertid lizards. *Ecology* **77**, 1163–1173.
- Bergmann, K. G. L. C. (1847). Über die verhältnisse der wärmeökonomie der thiere zu ihrer grösse. *Gött. Stud.* **3**, 595–708.
- Bittner, T. D., King, R. B. and Kerfin, J. M. (2002). Effects of body size and melanism on the thermal biology of garter snakes (*Thamnophis sirtalis*). *Copeia* **2002**, 477–482.
- Blouin-Demers, G. and Weatherhead, P. J. (2001). Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. *Ecology* **82**, 3025–3043.
- Burnham, K. P. and Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociol. Method. Res.* **33**, 261–304.
- Calbó, J., Pagès, D. and González, J.-A. (2005). Empirical studies of cloud effects on UV radiation: a review. *Rev. Geophys.* **43**, RG2002.
- Callaghan, T. V., Björn, L. O., Chernov, Y., Chapin, T., Christensen, T. R., Huntley, B., Ims, R. A., Johansson, M., Jolly, D., Jonasson, S. et al. (2004). Responses to projected changes in climate and UV-B at the species level. *AMBIO* **33**, 418–435.
- Carothers, J. H., Fox, S. F., Marquet, P. A. and Jaksic, F. M. (1997). Thermal characteristics of ten Andean lizards of the genus *Liolaemus* in central Chile. *Rev. Chil. Hist. Nat.* **70**, 297–309.
- Carrascal, L. M., López, P., Martín, J. and Salvador, A. (1992). Basking and antipredator behaviour in a high altitude lizard: implications of heat-exchange rate. *Ethology* **92**, 143–154.
- Castella, B., Golay, J., Monney, J.-C., Golay, P., Mebert, K. and Dubey, S. (2013). Melanism, body condition and elevational distribution in the asp viper. *J. Zool.* **290**, 273–280.
- Christian, K. A., Tracy, C. R. and Tracy, C. R. (2006). Evaluating thermoregulation in reptiles: an appropriate null model. *Am. Nat.* **168**, 421–430.
- Claussen, D. L. and Art, G. R. (1981). Heating and cooling rates in *Anolis carolinensis* and comparisons with other lizards. *Comp. Biochem. Physiol. A Physiol.* **69**, 23–29.
- Clusella-Trullas, S., van Wyk, J. H. and Spotila, J. R. (2007). Thermal melanism in ectotherms. *J. Therm. Biol.* **32**, 235–245.
- Clusella-Trullas, S., van Wyk, J. H. and Spotila, J. R. (2009). Thermal benefits of melanism in cordylid lizards: a theoretical and field test. *Ecology* **90**, 2297–2312.
- Cossins, A. R. and Bowler, K. (1987). *Temperature Biology of Animals*. New York: Chapman and Hall.
- Crisp, M., Cook, L. M. and Hereward, F. V. (1979). Color and heat balance in the lizard *Lacerta dugesii*. *Copeia* **1979**, 250–258.
- Cruz, F. B., Fitzgerald, L. A., Espinoza, R. E. and Schulte, J. A. II. (2005). The importance of phylogenetic scale in tests of Bergmann's and Rapoport's rules: lessons from a clade of South American lizards. *J. Evol. Biol.* **18**, 1559–1574.
- Díaz, J. A. (1991). Temporal patterns of basking behaviour in a Mediterranean lacertid lizard. *Behaviour* **118**, 1–14.
- Díaz, J. A., Bauwens, D. and Asensio, B. (1996). A comparative study of the relation between heating rates and ambient temperatures in lacertid lizards. *Physiol. Zool.* **69**, 1359–1383.

- Dzialowski, E. M. and O'Connor, M. P.** (1999). Utility of blood flow to the appendages in physiological control of heat exchange in reptiles. *J. Therm. Biol.* **24**, 21–32.
- Endler, J. A.** (1984). Progressive background in moths, and a quantitative measure of crypsis. *Biol. J. Linn. Soc.* **22**, 187–231.
- Espinoza, R. E., Wiens, J. J. and Tracy, C. R.** (2004). Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. *Proc. Natl. Acad. Sci. USA* **101**, 16819–16824.
- Fedorka, K. M., Lee, V. and Winterhalter, W. E.** (2013). Thermal environment shapes cuticle melanism and melanin-based immunity in the ground cricket *Allonemobius socius*. *Evol. Ecol.* **27**, 521–531.
- Fernández, J. B., Smith, J., Jr, Scolari, A. and Ibagüengoytia, N. R.** (2011). Performance and thermal sensitivity of the southernmost lizards in the world, *Liolaemus sarmientoi* and *Liolaemus magellanicus*. *J. Therm. Biol.* **36**, 15–22.
- Fitzgerald, L. A., Cruz, F. B. and Perotti, M. G.** (1999). Phenology of a lizard assemblage in the Dry Chaco of Argentina. *J. Herp.* **33**, 526–535.
- Forsman, A.** (1995). Heating rates and body temperature variation in melanistic and zigzag *Vipera berus*: does colour make a difference? *Ann. Zool. Fenn.* **32**, 365–374.
- Forsman, A.** (2011). Rethinking the thermal melanism hypothesis: rearing temperature and coloration in pygmy grasshoppers. *Evol. Ecol.* **25**, 1247–1257.
- Gaston, K. J. and Blackburn, T. M.** (2000). *Pattern and Process in Macroecology*. Malden: Blackwell Science.
- Gates, D. M.** (1980). *Biophysical Ecology*. New York: Springer.
- Gibson, A. R. and Falls, J. B.** (1979). Thermal biology of the common garter snake *Thamnophis sirtalis* L. II. The effects of melanism. *Oecologia* **43**, 99–109.
- Grigg, G. C. and Seebacher, F.** (1999). Field test of a paradigm: Hysteresis of heart rate in thermoregulation by a free-ranging lizard (*Pogona barbata*). *Proc. R. Soc. B Biol. Sci.* **266**, 1291–1297.
- Gunn, A.** (1998). The determination of larval phase coloration in the African armyworm, *Spodoptera exempta* and its consequences for thermoregulation and protection from UV light. *Entomol. Exp. Appl.* **86**, 125–133.
- Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. and Challenger, W.** (2008). GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131.
- Harris, R., McQuillan, P. and Hughes, L.** (2012). Patterns in body size and melanism along a latitudinal cline in the wingless grasshopper, *Phaulacridium vittatum*. *J. Biogeogr.* **39**, 1450–1461.
- Harvey, P. H. and Pagel, M. D.** (1991). *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.
- Heatwole, H. and Taylor, J. A.** (1987). *Ecology of Reptiles*. Chipping Norton, NSW: Surrey Beatty and Sons.
- Huey, R. B.** (1982). Temperature, physiology and the ecology of reptiles. In *Biology of the Reptilia 12* (ed. C. Gans and F. H. Pough), pp. 25–91. New York: Academic Press.
- Huey, R. B. and Pianka, E. R.** (1977). Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. *Ecology* **58**, 1066–1075.
- Huey, R. B., Hertz, P. E. and Sinervo, B.** (2003). Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* **161**, 357–366.
- Janse van Rensburg, D. A., Mouton, P. le F. N. and Van Niekerk, A.** (2009). Why cordylid lizards are black at the south-western tip of Africa. *J. Zool.* **278**, 333–341.
- Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P. and Webb, C. O.** (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463–1464.
- Kettlewell, H. B. D.** (1973). *The Evolution of Melanism: The Study of a Recurring Necessity, with Special Reference to Industrial Melanism in the Lepidoptera*. Oxford: Clarendon Press.
- Kingsolver, J. G. and Wiernasz, D. C.** (1991). Development, function, and the quantitative genetics of wing melanin pattern in *Pieris* butterflies. *Evolution* **45**, 1480–1492.
- Labra, A., Pienaar, J. and Hansen, T. F.** (2009). Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. *Am. Nat.* **174**, 204–220.
- Lillywhite, H. B.** (1980). Behavioral thermoregulation in Australian elapid snakes. *Copeia* **1980**, 452–458.
- Lindstedt, C., Lindström, L. and Mappes, J.** (2009). Thermoregulation constrains effective warning signal expression. *Evolution* **63**, 469–478.
- Martins, E. P.** (1996). Conducting phylogenetic comparative analyses when the phylogeny is not known. *Evolution* **50**, 12–22.
- Moreno Azócar, D. L.** (2013). Relaciones entre distribución geográfica, melanismo y tamaño corporal en lagartijas *Liolaemus* del grupo goetschi. Re- evaluación de la regla de Bergmann, patrones y procesos. *PhD dissertation*. Universidad Nacional del Comahue, Río Negro, Argentina.
- Moreno Azócar, D. L., Vanhooydonck, B., Bonino, M. F., Perotti, M. G., Abdala, C. S., Schulte, J. A., II and Cruz, F. B.** (2013). Chasing the Patagonian sun: comparative thermal biology of *Liolaemus* lizards. *Oecologia* **171**, 773–788.
- Moreno Azócar, D. L., Perotti, M. G., Bonino, M. F., Schulte, J. A., II, Abdala, C. S. and Cruz, F. B.** (2015). Variation in body size and degree of melanism within a lizards clade: is it driven by latitudinal and climatic gradients? *J. Zool.* **295**, 243–253.
- Norris, K. S.** (1967). Color adaptation in desert reptiles and its thermal relationships. In *Lizard Ecology: a Symposium* (ed. W. W. Milstead), pp. 162–229. Columbia: University of Missouri Press.
- Olalla-Tárraga, M. Á. and Rodríguez, M. Á.** (2007). Energy and interspecific body size patterns of amphibian faunas in Europe and North America: anurans follow Bergmann's rule, urodeles its converse. *Global Ecol. Biogeogr.* **16**, 606–617.
- Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N. and Pearse, W.** (2013). Caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.2. Available at <http://CRAN.R-project.org/package=caper>.
- Paradis, E., Claude, J. and Strimmer, K.** (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290.
- Pereboom, J. J. M. and Biesmeijer, J. C.** (2003). Thermal constraints for stingless bee foragers: the importance of body size and coloration. *Oecologia* **137**, 42–50.
- Pinheiro, J., Bates, D., DebRoy, S. and Sarkar, D. and R Core Team** (2015). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–119. Available at <http://CRAN.R-project.org/package=nlme>.
- Porter, W. P.** (1967). Solar radiation through the living body walls of vertebrates with emphasis on desert reptiles. *Ecol. Monogr.* **37**, 273–296.
- Porter, W. P. and Gates, D. M.** (1969). Thermodynamic equilibria of animals with environment. *Ecol. Monogr.* **39**, 227–244.
- Porter, W. P. and Tracy, C. R.** (1983). Biophysical analyses of energetics, time-space utilization, and distributional limits. In *Lizard Ecology: Studies of a Model Organism* (ed. R. B. Huey, E. R. Pianka and T. W. Schoener), pp. 55–83. Cambridge: Harvard University Press.
- Ragland, G. J. and Kingsolver, J. G.** (2008). The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyomyia smithii*. *Evol. Ecol. Res.* **10**, 29–44.
- R Core Team** (2014). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org/>.
- Reguera, S., Zamora-Camacho, F. J. and Moreno-Rueda, G.** (2014). The lizard *Psammotromus algirus* (Squamata: Lacertidae) is darker at high altitudes. *Biol. J. Linn. Soc.* **112**, 132–141.
- Revell, L. J.** (2010). Phylogenetic signal and linear regression on species data. *Methods Ecol. Evol.* **1**, 319–329.
- Revell, L. J.** (2012). Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223.
- Roff, D. A. and Fairbairn, D. J.** (2013). The costs of being dark: the genetic basis of melanism and its association with fitness-related traits in the sand cricket. *J. Evol. Biol.* **26**, 1406–1416.
- Seebacher, F.** (2000). Heat transfer in a microvascular network: the effect of heart rate on heating and cooling in reptiles (*Pogona barbata* and *Varanus varius*). *J. Theor. Biol.* **203**, 97–109.
- Seebacher, F. and Grigg, G. C.** (2001). Changes in heart rate are important for thermoregulation in the varanid lizard *Varanus varius*. *J. Comp. Physiol. B* **171**, 395–400.
- Shine, R. and Kearney, M.** (2001). Field studies of reptile thermoregulation: how well do physical models predict operative temperatures? *Funct. Ecol.* **15**, 282–288.
- Smith, E. N.** (1976). Heating and cooling rates of the American alligator, *Alligator mississippiensis*. *Physiol. Zool.* **49**, 37–48.
- Stevenson, R. D.** (1985). The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* **126**, 362–386.
- Strugariu, A. and Zamfirescu, S. R.** (2011). Population characteristics of the adder (*Vipera berus berus*) in the Northern Romanian Carpathians with emphasis on colour polymorphism: is melanism always adaptive in vipers? *Anim. Biol.* **61**, 457–468.
- Tanaka, K.** (2007). Thermal biology of a colour-dimorphic snake, *Elaphe quadrivirgata*, in a montane forest: do melanistic snakes enjoy thermal advantages? *Biol. J. Linn. Soc.* **92**, 309–322.
- Tanaka, K.** (2009). Does the thermal advantage of melanism produce size differences in color-dimorphic snakes? *Zool. Sci.* **26**, 698–703.
- Turner, J. S.** (1987). The cardiovascular control of heat exchange: consequences of body size. *Am. Zool.* **27**, 69–79.
- Turner, J. S. and Tracy, C. R.** (1983). Blood flow to appendages and the control of heat exchange in American alligators. *Physiol. Zool.* **56**, 195–200.
- Warnes, G. R., Bolker, B. and Lumley, T.** (2014). Gtools: Various R programming tools. R package version 3.4.1. Available at <http://CRAN.R-project.org/package=gtools>.
- Watt, W. B.** (1968). Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation of melanin pigment in relation to thermoregulation. *Evolution* **22**, 437–458.
- Wickham, H.** (2009). *Ggplot2: Elegant Graphics for Data Analysis*. New York: Springer.
- Wiernasz, D. C.** (1989). Female choice and sexual selection of male wing melanin pattern in *Pieris*. *Biol. Lett.* **4**, 637–649.
- Zamora-Camacho, F. J., Reguera, S. and Moreno-Rueda, G.** (2014). Bergmann's Rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-metre elevational gradient. *J. Evol. Biol.* **27**, 2820–2828.