Intraspecific variation in the energetics of the Cabrera vole

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ABSTRACT

Basal metabolic rate (BMR) is an intensively topic studied in ecophysiology for the purpose of understanding energy budgets of the species, variations of energy expenditure during their diary activities and physiological acclimatization to the environment. Establishing how the metabolism is assembled to the environment can provide valuable data to improve conservation strategies of endangered species. In this sense, metabolic differences associated to habitats have been widely reported in the interspecific level, however little is known about the intraspecific view of BMR under an environmental gradient. In this study, we researched the effect of the habitat on metabolic rate of an Iberian endemic species: Iberomys cabrerae. Animals were captured in different subpopulations of its altitudinal range and their MR was studied over a thermal gradient. MR was analyzed through a Linear Mixed Model (LMM) in which, in addition to thermal effects, the bioclimatic zone and sex also influenced the metabolism of the species. The beginning of thermoneutrality zone was set on 26.5 °C and RMR was 2.3 ml $O_2 \, g^{-1} \, h^{-1}$, intermediate between both bioclimatic zones. Supramediterranean subpopulations started the T_{lc} earlier (24.9 °C) and had higher RMR than the mesomediterranean ones (26.9 °C). The thermal environment together with primary productivity conditions could explain this difference in the metabolic behaviour of the Cabrera voles.

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1. Introduction

Macrophysiological patterns and its variability in time and space are of vital importance to gain insight about evolutionary and ecological theories (Chown and Gaston, 2008; Naya et al., 2013; Bozinovic et al., 2014). In addition, the relatively recent trend in niche modelling literature has evolved from correlative models relating the presence of species to niche conditions to mechanistic models based on physiological data (Kearney and Porter, 2009). Despite the correlative approach is widely used (e.g. Anderson and Raza, 2010; Zeng et al., 2015) it shows low predictive power when is used to predict abundance and distribution over novel environmental conditions and it cannot explain the fundamental causation of geographical distribution (Kearney and Porter, 2004), which could be satisfied with the mechanistic approach. Mechanistic models integrate behavioural, morphological and physiological traits of the organism with the habitat features using energy and mass balance equations to determinate the species' range (Barve et al., 2014). In this field, the energetic metabolism is an intensively studied topic (e.g. Rezende et al., 2004; Agosta et al., 2013), owing to some of its traits as the metabolic rate responses directly to climate conditions; it can be related to the fundamental niche and then be used to model distribution and abundance or predicting potential changes linked to novel climate conditions.

Among various physiological measurements of ecological relevance, basal metabolic rate (BMR) represents the minimum rate of energy necessary to maintain homeostasis and allostasis. BMR has been used as a standard to assess the costs of different components of organism energy budgets, to analyze species-specific as well as intraspecific variations in energy expenditure during maximal and sustained activities, and to understand physiological adaptations to the environment (McNab, 2002). The dependence of metabolic rates on body mass (M_b) has long been recognized (Kleiber, 1961). Nevertheless, M_b alone does not fully explain variation in BMR (McNab, 1992). There are several hypotheses that attempt to explain how biotic and abiotic conditions affect mass-independent BMR in mammals (McNab, 2002), namely, food quality, food availability and/or unpredictable, direct climate effects, aridity, among others. For instance, several interspecific studies have analyzed the effects of food availability and predictability on mass-independent BMR by demonstrating that mass-independent BMR is higher in mesic habitats when compared with xeric habitats

(Lovegrove, 2000; Mueller and Diamond, 2001; McNab, 2002; Rezende et al., 2004; Bozinovic et al., 2007). Among rodent species it has been largely reported the clear association between the mass corrected-metabolism rate and several key abiotic factors, such as: latitude (Speakman, 2000; Lovegrove, 2003; Rezende et al., 2004), altitude (Hayes, 1989), or climate (McNab, 1970; Hulbert et al., 1985; Bozinovic and Rosenmann, 1989). Most of these studies evaluated the effect of the environment on BMR from an interspecific level approach, considering that the traits are fixed and thus the BMR value is unique for each species. Nevertheless, some genetic or plastic variability can exist so the analysis of the metabolism rates focuses on the intraspecific level which allows approximating the factors responsible for variations in BMR and the ability of the species to adapt to the environment (Cruz-Neto and Bozinovic, 2004). Therefore, studies on changes in BMR across environmental gradients can help to predict the potential response of the species to anthropogenic changes in the environment and then to design better strategies for their conservation.

We investigated here the effects of geographic variations in habitat on the energetics (i.e. RMR, minimal thermal conductance (Cm) and minimal temperature differential between body (Tb) and environment (Ta) $= \Delta$Tm) of rodents originated from different populations. We used as study model populations of *Iberomys cabrerae* (*Microtus cabrerae* see Cuenca-Bescós et al., 2013) which is an endemic vole of the Iberian Peninsula, classified as Near Threatened in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Fernandes et al., 2008). The species has a highly fractured distribution area that is restricted to the centre, northeast and southeast of the Peninsula (Garrido-García et al., 2013). The Cabrera vole is a specialist species of scenopoetic variables with very restrictive habitat preferences. It has a patchy distribution in open areas with high water table and covered by formations of perennial grasses and rush beds. The subpopulations are distributed along an altitudinal range from 250 to 1500 m, although they are more common in meso- and supramediterranean bioclimatic zones, between 500 and 1200 m (Fernández-Salvador, 1998). Because of its habitat specificity and limited geographical distribution, the species is highly vulnerable to anthropic activities such as agriculture, farming or human infrastructures (Landete-Castillejos et al.,

2000). Furthermore, predictions on climate change in the Iberian Peninsula expect an increase in the xericity (Pachauri et al., 2014) which could change the suitability of Cabrera voles' habitats compromising its conservation and persistence.

Mathias et al. (2003) and Santos et al. (2004) have explored the energetic metabolism of *I. cabrerae* but ignoring the intraspecific approach of this physiological trait. Consequently, our main aim is to evaluate the energetics of Cabrera vole at an intraspecific level in the Cabrera vole and to test for interpopulation differences associated to bioclimatic conditions.

2. Materials and methods

2.1. Experimental animals

This study is made up of 15 adults of *I. cabrerae*, from different subpopulations of two bioclimatic zones: mesomediterranean (subpopulations between 480 m and 710 m a.s.l.) and supramediterranean (subpopulations between 900 m and 1050 m a.s.l.) in Madrid, Spain (Fig. 1). A total of 8 animals were captured for mesomediterranean bioclimatic zone (5 females and 3 males) and 7 in the supramediterranean (4 females and 3 males). Cabrera voles were captured using Sherman live traps supplied with apples, during the day because this species shows diurnal and nocturnal activity (Fernández-Salvador, 1998). Due to the randomness of trapping, it was not possible to obtain equal number of male and female from each bioclimatic range. *I. cabrerae* is a Near Threatened species (Fernandes et al., 2008), thus we had to reach a compromise between selecting a representative number of voles and not affecting negatively to the sampled subpopulations, so we had to use a reduced sample of experimental animals.

Trapping was conducted in four different subpopulations in the supramediterranean zone and in six subpopulations in the mesomediterranean, during June and July of 2013 (Fig. 1). The annual average temperature in subpopulations of the supramediterranean zone varies between 11.1° and 11.9 °C; with maximum temperatures in July between 21.2° and 21.9 °C; minimum temperatures in January ranging from 2.8° to 3.4 °C; the minimum rainfall values occurring in
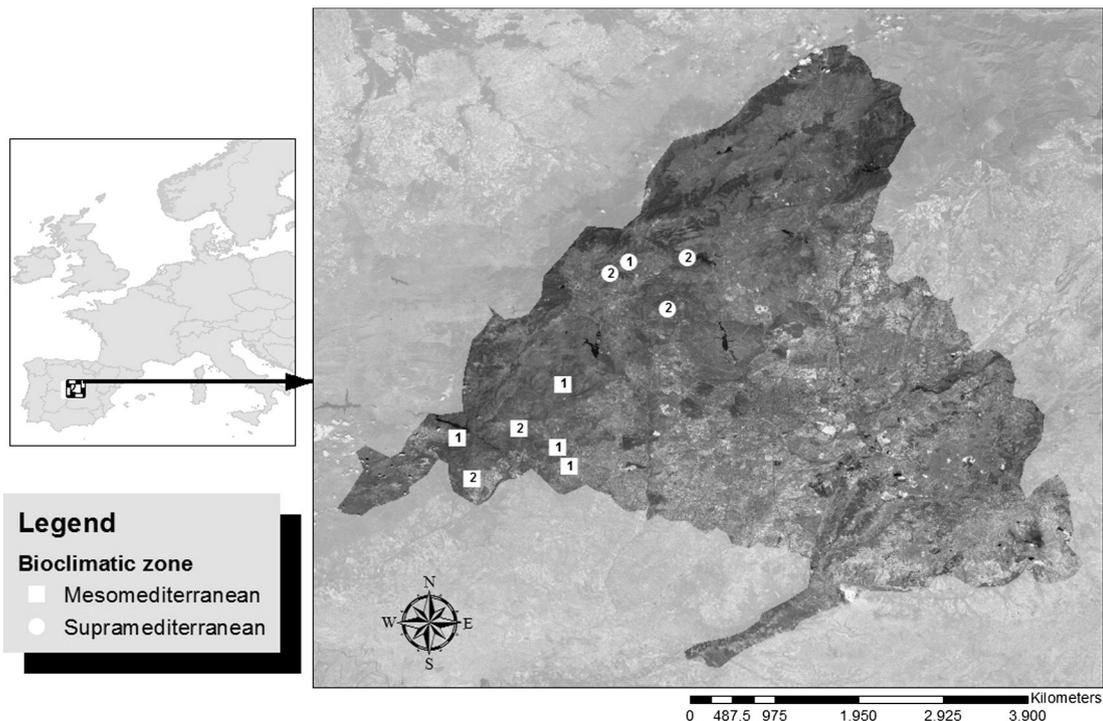

Fig. 1. Geographical localization of the subpopulations where the Cabrera voles were taken for the study. Numbers in the symbol are individuals captured in the respective subpopulation.

August (14 and 15 mm); and total rainfalls of the area varying from 472 to 484 mm. Whereas in the subpopulations of the mesomediterranean zone the annual average temperature varies between 13° and 14.6 °C; the maximum temperatures occur in July with values from 23.3° to 24.9 °C; the minimum temperatures in January, range from 4.4° to 5.7 °C; the minimal rainfalls happen in July, from 9 to 12 mm; and total rainfalls range from 370 to 419 mm (AEMET, 2015). These climate differences between bioclimatic zones involve differences in the configuration and composition of the vegetation of the colonies. Supramediterranean subpopulations are large areas made by dense structures of *Stipa gigantea*, and sometimes they also include patches of *Agrostis castellana*. However, in the mesomediterranean range subpopulations occupy smaller areas with a continuous canopy of *A. castellana* and scattered rushes (*Juncus* spp.).

The effect on primary productivity is a higher averaged value of the Normalized Difference Vegetation Index (NDVI), a proxy of primary productivity (Pettorelli et al., 2005), for supramediterranean areas, although the difference between areas was statistically marginal (ANOVA; $F_{1,8} = 4.65$; $p = 0.06$), we observed ecological differences between habitats. The NDVI values for subpopulations were derived from MOD13Q1 NDVI, a satellite image captured by the MODIS sensor aboard the NASA's TERRA satellite (https://lpdaac.usgs.gov/products/) and processed with ArcGIS tools. Only one satellite image was downloaded corresponding to the voles' capture date (June of 2013), with a spatial resolution of 231 m × 231 m. This is the minimum spatial resolution image that allowed us to cover the NDVI variability for the size of the subpopulations evaluated (from 500 m^2 to 47,000 m^2).

Trapping event was performed between June and July of 2013. Each captured animal was transported to the laboratory on the same day of capture and its metabolism rate was measured within 24 h. The animals were distributed in individual transparent cages (40 × 26 × 27 cm) accommodated with hay to simulate vole's habitat and fed with apple and oat ad libitum (Fernández-Salvador et al., 2001). Finally, all voles were released alive in their respective subpopulations. All animal procedures were approved by the Animal Ethics Committee of The Rey Juan Carlos University of Madrid (Spain).

2.2. Metabolic rates

Metabolic rate of *I. cabrerae* was measured as the rate of oxygen consumption (VO₂), using a computerized open-flow respirometry system (Sable Systems, Anderson, NV). Animals were situated inside a metabolic chamber (volume, 1.3 L) that was provided with a metallic grid which supports the voles to isolate them from their own faeces and allows them to adopt a relaxed posture during measurements. The size of the chamber was a compromise between ensure some animal movement but small enough to prevent excessive activity. The metabolic chamber was pumped with a flow of 800 ml min^{-1} of dried atmospheric air which is enough to ensure the mixture inside. On the entry and the outlet of the metabolic chamber were placed a CO_2-absorbent filter of Soda Lime and a H_2O-absorbent filter of Drierite, arranged in series, so avoiding the CO_2 and H_2O influence in the O_2 measures. The output flow was sampled each 5 s by the FoxBox Oxygen Analysis System (Sable Systems, Anderson, NV), and the oxygen consumption was analyzed with the ExpeData data acquisition software (http://www.sablesys.com) following the equation of Withers (1977):

$$Vo_2 = \frac{V_E \cdot (FI_{O2} - FE_{O2})}{1 - FI_{O2}}$$

where Vo₂ is the rate of O₂ consumption; V_E is the rate of airflow pumped into the metabolic chamber; FI_{O2} is the fractional concentration of O₂ entering the metabolic rate; and FE_{O2} is the fractional concentration of O₂ leaving the metabolic rate. Cabrera voles were measured at different ambient temperatures ranging from 5° to 36 °C (ca. 4 h). At each temperature the metabolic rate was taken as the minimal oxygen consumption at least during 3 min. Metabolic rates were expressed as mass-specific metabolic rates (ml O₂ g^{-1} h^{-1}). Since *I. cabrerae* is a near threatened species we did not want to jeopardize it, so we delimited the maximum temperature which submit voles to 36 °C. Within the thermoneutrality range, the metabolic rates were measured as resting metabolic rates (RMR) because animals were not under fasting conditions. Minimal thermal conductance (Cm) was calculated as the slope of the metabolism–environmental temperature curve under cool conditions (ml O₂ g^{-1} h^{-1} °C^{-1}). Voles were weighted with an electronic scale (± 0.1 g) before and after VO₂ records.

Resting metabolic rate was compared with the predicted standard rate of metabolism for eutheria: BMR $= 3.42 \times M_b^{-0.25}$ (Kleiber, 1961), small grazers: BMR $= 7.65 \times M_b^{-0.38}$ and BMR $= 4.18 \times M_b^{-0.32}$ (McNab, 1988). In addition we compared the observed Cm against the expected one based on Cm $= 1.0 \times M_b^{-0.50}$ (Morrison and Ryser, 1951). According to Cooper and Withers (2006) the 95% prediction confidence intervals were calculated for each one of these allometric regressions. We followed this approach to evaluate statistically if the Cabrera vole's energetic traits (RMR and Cm) were closed to the expected values based on M_b. The minimal (body-ambient) thermal differential (ΔTm) was calculated in each habitat and was also used to estimate the theoretical lower critical temperature of thermoneutrality (T_{Ic}^*) for the species following the McNab's (1974) equation ($\Delta Tm = T_b - T_{Ic} = $ BMR / Cm or $\Delta Tm = T_b - T_{Ic} = 3.42 \times M_b^{0.25}$).

2.3. Statistical analysis

All statistical analyses were performed using R software (R Core Team, 2014) through suitable packages for linear mixed models (LMM): nlme (Pinheiro et al., 2014) and lme4 (Bates et al., 2014) with normal response distribution for metabolic rate variable. To evaluate the variability over the metabolic rate we design a model where sex, bioclimatic zone, temperature and individuals were tested. The model was composed by the metabolic rate as the response variable with continuous range, the bioclimatic zone and sex were fixed factors with two levels respectively as environmental temperature was also a fixed factor with 12 levels to cover the range from 5° to 36 °C. Voles were included in the model as random effects to control individual conditions and to generalize the results to Cabrera vole's populations. The structure of random effects was optimized following the protocol outlined in Zuur et al. (2009), considering the contribution of fixed factors to modify the intercept and/or slopes of functional relationship between RMR and individuals. According to Bolker et al. (2009) and Zuur et al. (2009) in each model the parameter estimation of fixed effects was done under maximum likelihood (ML) method, while the final model estimation of the parameters which better described the metabolic response of *I. cabrerae* was based on restricted maximum likelihood (REML). Thus, it was designed a battery of 19 models in which the goodness of fit to the dataset was assessed with the corrected Akaike Information Criterion (AICc). Better models were selected from the model set considering as good models they which did not differ in more than 2 units of AICc ($\Delta AICc$) from the model with the lower AICc (Burnham and Anderson, 2002) thanks to the functions of the package MuMIn (Barton, 2014). *Model average* function from MuMIn package was used to get the only one best model, so its model coefficients were averaged according to the relative importance of each independent variable and the number of models containing those variables. Finally the structure of the residuals was evaluated to corroborate the good fit of the selected model to the dataset.

The identification of the thermoneutrality zone was conducted based on piecewise regression thresholds to reveal the point of change. Piecewise regressions are widely employed for modelling ecological relationships defined by abrupt changes in the response variable when an ecological threshold is exceeded (Rhodes et al., 2008; Ficetola and Denoël, 2009). The beginning of thermoneutral zone implies a change in the slope of the regression curve of metabolic rate, thus the piecewise

regression threshold analysis was focused on finding a relevant change in that slope. Intervals for the breaking points of the piecewise regression threshold were defined inside the linear mixed model with the function *lmer*.

3. Results

The metabolic response of the *I. cabrerae* to the range of their environmental temperatures was explained through a linear mixed model that included temperature, sex and bioclimatic zone as fixed factors and a random effect over the individual with a variable effect depending on the temperature. Only one better model was chosen to explain the metabolism differences in the subpopulations of Cabrera vole (Table 1). The selected model explained the 88% of the data variance, in which 77% was assumed by fixed factor and only the 11% of the variance was a consequence of individuals.

The relationship between MR and temperature followed the general trend of endothermic homeotherm animals with a change rate of −0.126 and a random variance of 0.001 associated to the individual response while the temperature increased (Table 2).

Under the whole temperature cycle, the average of metabolic rate was higher for supramediterranean voles than mesomediterranean (Fig. 2). The model estimates a metabolic rate for mesomediterranean zone that is summarized in the intercept value (Table 2) over which there is an increment of 0.509 (±0.332) for explaining the metabolic rate of supramediterranean zone.

Sex differences had also influence over the metabolic rate voles studied. Males from both bioclimatic zones had lower metabolic rates, on average they had a value of −0.348 (±0.319) over the intercept (Table 2). In contrast, females had upper rates, which increased in 0.509 (±0.332) for females from supramediterranean zone and just the intercept value for mesomediterranean zone females.

The individual metabolic response of *I. cabrerae* to the range of ambient temperatures allowed defining the zone of thermoneutrality for the species (Fig. 2). At low ambient temperatures the metabolic rate was high and decreased as the temperature increased until 26.5 °C when the rate tended to stability and thus TNZ started then. In the temperature range above the starting point of TNZ, the metabolic rate tended to the resting metabolic rate. Metabolic variables for subpopulations of each bioclimatic zone studied are compiled in Table 3. The upper limit of the TNZ could not be determined to avoid any death.

The percentage of change of metabolic traits measured in our study in comparison to the predicted values from the allometric equations of Kleiber (1961), McNab (1988) and Morrison and Ryser (1951) are summarized in Table 3. The Cabrera vole's RMR for the global species and the ones analyzed by bioclimatic zones were higher than the expected from M_b and also fell out the 95% of the predicted confidence intervals for the three allometric regression (1.241–1.549 (Kleiber, 1961); 1.466–1.956 and 1.117–1.253, for small grazer mammals and Rodentia mammals, respectively (McNab, 1988)). Also, and regarding thermal conductance, values of the species and subpopulations were higher than those predicted from the mass (Morrison and Ryser, 1951) and were outside the 95% predicted confidence interval (0.120–0.137).

4. Discussion

Levels of metabolic rates in mammals are influenced by different factors, including body mass, phylogenetic relatedness, activity, biotic and abiotic habitat conditions, and food habits among others (McNab, 2002). For example, Mueller and Diamond (2001) and Bozinovic et al. (2007) found a high-BMR in rodent species and populations from high-productivity environments in comparison to species and populations from low-productivity habitats. These authors concluded that BMR is driven by NPP and, hence, food availability.

Cabrera vole is a robust species with higher body mass than similar voles of the genus *Microtus* (Fernández-Salvador, 1998). Despite *I. cabrerae* are relatively heavy voles, its mass-specific RMR values are relatively lower than the values reported for other arvicolines species (Cricetidae, Rodentia) such as *Chionomys nivalis* (35 g), *Microtus agrestis* (22 g) (McNab, 1986), *Microtus arvalis* (30 g) (Hart, 1971), *Microtus oeconomus* (32 g) (Lantova et al., 2011), *Microtus maximowiczii* (38 g) (Chen et al., 2012) or *Clethrionomys glareolus* (23 g) (Aalto et al., 1993; Labocha et al., 2004). However, average values obtained in this study (2.278 ± 0.797 ml O$_2$ g^{-1} h^{-1}) are higher than the ones reported by Mathias et al. (2003) for the same species but situated in lower altitudes in Portugal. In addition to the altitude differences, the Atlantic influence on climatic conditions and grass vegetation of the Portuguese populations could produce particular habitats which reduce the metabolic rate, maybe implying lower habitat quality for this population (e.g. food quality hypothesis, Bozinovic et al., 2007; Bozinovic et al., 2009).

Against the theoretical metabolism predicted by Kleiber (1961), our results reveal a higher resting metabolic rate which underscores that the metabolism is also contributed by ecological factors (Rezende et al., 2004; Luna et al., 2009). Alike, our outcomes exceed the BMR predicted for small grazer mammals or for mammals from order Rodentia (McNab, 1988). Small grazer mammals as microtines have higher basal metabolic rates than the expected for their body mass, as a consequence of their food habits (grazing evergreen food), their terrestrial behaviour that requires high levels of energy to escape from predators or as result of the climate in their habitats (McNab, 1986). Furthermore, the BMR level is also contributed by the taxonomic affiliation (Hayssen and Lacy, 1985) so, the BMR averaged of species from genus *Microtus* is a 200.6% higher than the BMR expected for species of order Rodentia (McNab, 1988), hence that an exceeded level of RMR over the predicted was expected for Cabrera voles.

In the same way, the thermoneutrality zone defined for *I. cabrerae* in our study ranges from 26.5° to 36 °C, which makes the beginning of TNZ early with respect of the 30 °C identified by Mathias et al. (2003) for this species. Differences between both values are attributed to local conditions of the analysis which is why TNZ cannot be extrapolated to any environment (Romanovsky et al., 2002) or because TNZ can be affected mainly by differences in habitat quality, productivity or climate. The maximum threshold of measured temperature was 36 °C, for avoiding any animal dead, hence we cannot determine the upper limit of the thermoneutral zone; however we suppose that it is closed to the upper critical temperature of TNZ, because upper lethal temperature is often 3°–6 °C above the normal body temperature (36.6 °C,

Table 1
Summary of the four best linear mixed models to explain the metabolic rate of Cabrera voles. In bold is highlighted the best model that was the only selected model.

Model	T	BZ	S	BZ:S	S:T	k	logLik	ΔAICc	w_i	R^2m	R^2c
1	**X**	**X**	**X**	**X**		**5**	**−143.14**	**0.00**	**0.39**	**0.77**	**0.88**
2	X	X	X			4	−144.52	0.53	0.30		
3	X		X			3	−146.27	1.83	0.16		
4	X	X	X	X	X	6	−143.00	1.99	0.15		
Parameter weights	1.00	0.84	1.00	0.54	0.15						

k is the number of parameters in the model; ΔAICc is the difference in AICc between each model with the best model (with lower AIC); w_i is the AICc weight of the average model. The fixed parameters in the model are included by their initials (T: temperature; BZ: Bioclimatic zone; S: Sex). The R^2c is the conditional R^2 which is referred to the global model while R^2m is the marginal R^2 that is only bounded to fixed effects of the model. These last parameters were only expressed for the selected model. *Parameter weights* reveal the relative importance of each variable depending on the number of containing models in which they are included after analyzing the models with the model average function.

Table 2
Parameters estimated by the selected model to explain the metabolic response of *I. cabrerae*.

Fixed effects					Random effects			
Estimate	Intercept	T	BZs	Sm	BZs:Sm	Variance	Individual	T	Residual
	6.217	−0.126	0.509	−0.348	−0.381		0.607	0.001	0.254
SE	0.284	0.008	0.332	0.319	0.448	SD	0.779	0.027	0.504

T is the temperature factor, BZ is the bioclimatic zone and S is the sex, BZ:S indicates the interaction between both factors. In the model's output the estimate summarizes the mesomediterranean level of the factor Bioclimatic zone (BZ), the female level of the factor Sex (S) is also included in the intercept, as well as the interaction between the levels mesomediterranean and female sex. Thus, the fixed factor bioclimatic zone only expressed the estimate for the supramediterranean level (BZs), the sex factor only included the parameters for males (Sm) while the interaction between both factors considered the same levels (BZs:Sm).

Castellanos-Frías et al., unpublished data) (Willmer et al., 2005). The T_{lc} is closed to the theoretical T_{lc}^{*} which situates the beginning of TNZ in 27.36 °C, only 0.9 °C warmer than that measured in this study. Our results support data from other arvicolines species, the Cabrera vole's TNZ covers a slightly warmer and wider temperature range than that of other species as *M. agrestis* from 25° to 30 °C (McDevitt and Speakman, 1994), *M. maximowiczii* from 25° to 32.5 °C (Chen et al., 2012) or *C. glareolus* with a range from 25° to 30 °C (Aalto et al., 1993). The intraspecific analysis reveals an earlier beginning in TNZ for supra-populations (T_{lc} = 24.85 °C) than in mesomediterranean (T_{lc} = 26.85 °C), this pattern of advance in temperature of TNZ might be related to colder thermal environments (Willmer et al., 2005).

In general, the low value of resting metabolic rate could manifest a selective advantage of low metabolic rates in habitats with low-medium trophic quality (Louw and Seely, 1982), because the minimal energetic requirements are reduced (Veloso and Bozinovic, 1993) and then animals can survive with lower food supply. Summer conditions in Mediterranean areas define food restrictions and unpredictability in Cabrera voles' habitats. Droughts joined high temperatures promote the reduction of trophic resources available (e.g. green grasses, Bozinovic et al., 2009), thus summers act as a bottleneck regulating their populations and selecting towards minimal rates. Ventura et al. (1998) and Mathias et al. (2003) suggested that there could be a seasonal different response in body mass and basal metabolic rate of *I. cabrerae*, however this study has not considered that variance. The aridity increase of Mediterranean climate is one of the main short time threats with which this species has to face to, thus we studied the restrictive period in the species and focused on spring–summer when the climatic conditions could question the survival of the individuals (Fernández-Salvador et al., 2005).

Metabolic differences assigned to altitude could be mainly attributable to the effect of the thermal environment and the primary production of habitat, since the difference of altitudinal is not large enough to
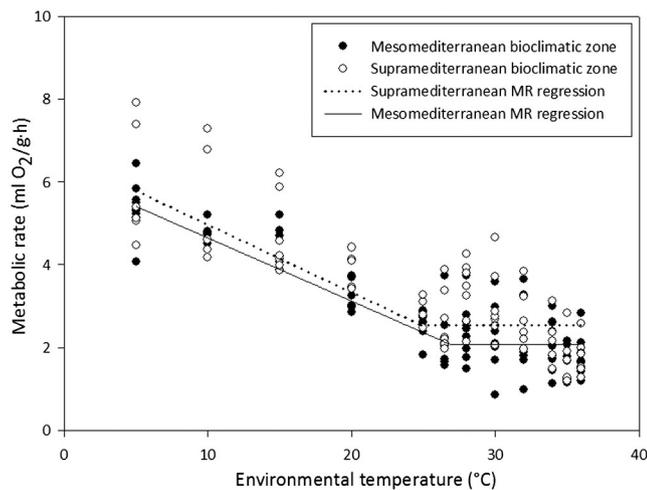
account for other effects (e.g. P_{O_2} or radiation load). The thermal environment contributes significantly to the variance in metabolic rate between species, thus Bozinovic and Rosenmann (1989) found that the cold-induced maximum metabolic rate (MMR) was higher in rodents from cold climates, while tropical species showed tendency to lower values. Under low temperatures, the metabolic rates of the endothermic animals increase as a consequence of their thermoregulatory costs (McNab, 2002), hence the predominance of cooler conditions at higher altitudes arises as the basis for differences in RMR between supra- and mesomediterranean subpopulations. Along the same lines, the strategies to keep or dissipate the heat are adapted to the thermal environment (Willmer et al., 2005), nevertheless, in spite of the thermal contrast in the areas of study, no major differences occur in the conductance value between individuals from both bioclimatic zones. The same pattern is kept in the species' conductance that is slightly lower than Mathias' et al. (2003) average (0.152 ± 0.008 ml O_2 g^{-1} h^{-1} $°C^{-1}$). The robustness in the magnitude of thermal conductance is an expected result, since it depends mainly on the body mass (Morrison and Ryser, 1951) and there is not body weight differences between samples of both studies, little differences are due to the temperature differential between the animal and its environment. Thus, compared to the theoretical conductance, our outcome is lightly higher (111%) than the predicted value for this species. A review by bioclimatic levels reveals that mesomediterranean subpopulations have a thermal conductance higher than the supramediterranean ones, reflecting the greater need of meso-subpopulations to dissipate heat. Consequently, ΔTm is wider for supra- than mesomediterranean subpopulation, hence supramediterranean individuals have a little wider range of thermal tolerance for which the metabolic rate is the minimum, and thus advantageous for this subpopulation.

Regarding primary production of habitats, both bioclimatic zones differ in the vegetation composition, which could contribute to differences between bioclimatic zones, so the supramediterranean subpopulations were mainly constituted by *S. gigantea* and sometimes by *A. castellana* while the mesomediterranean only had *A. castellana*. According to the *Food Quality Hypothesis* (Cruz-Neto and Bozinovic, 2004; Bozinovic et al., 2007, 2009) BMR values are associated with the diet, thus the variability of habitats in food quality, availability and predictability leads to differences in BMR into the species. Despite the nutritional component of habitats was not evaluated in the study, however values of NDVI for summer period showed slightly higher productivity in the supramediterranean than in the mesomediterranean range, this difference was marginally non significant statistically. Because the number of subpopulations sampled was small to compare NDVI values between bioclimatic ranges, a higher number of subpopulations would be necessary in order to improve the knowledge of the effect of habitat quality in BMR.

Differences in metabolic rate are also relevant between sexes. The basic energetic threshold required by females was higher than males under both bioclimatic levels. Similar result was found by Boratyński and Koteja (2009) and Boratyński et al. (2010) with wild populations of *Myodes glareolus*. Reproduction is one of the most costly processes in mammals (Harvey, 1986; Gittleman and Thompson, 1988), therefore a parental provisioning could be profitable to ensure the reproduction success. In this way, when females have an elevated minimum

Fig. 2. Individual metabolic rate of *Iberomys cabrerae* from different bioclimatic zones in response to ambient temperatures. The change in the slope of the curve reveals the beginning of the thermoneutral zone for each bioclimatic zone.

Table 3
Body mass (M_b), resting metabolic rate (RMR), lower critical temperature of thermoneutrality (T_{lc}), theoretical lower critical temperature of thermoneutrality (T_{lc}^*), thermal conductance (Cm) and minimal (body-ambient) thermal differential (ΔTm) for the global species and the subpopulations of *I. cabrerae*.

	M_b (g)		RMR (ml O_2/g h)		T_{lc} (° C)	T_{lc}^* (°C)	Cm (ml O_2 g^{-1} h^{-1} $°C^{-1}$)	ΔTm (° C)	% BMR	% BMR (small grazers)	% BMR (order Rodentia)	% Cm
	Mean	sd	Mean	sd					Kleiber (1961)	McNab (1988)		Morrison and Ryser (1951)
Iberomys cabrerae	51.16	6.09	2.278	0.797	26.49	27.36	0.142	15.9	177.8%	134.4%	191.2%	110.9%
Supramediterranean subpopulation	51.19	5.52	2.539	0.819	24.85		0.138	18.4	198.2%	149.8%	213.1%	107.7%
Mesomediterranean subpopulation	51.14	6.58	2.078	0.694	26.85		0.146	14.2	162.2%	122.6%	174.4%	113.8%

metabolism, this suggests that they have a higher energy potential to deal with the maternity and the breeding of offspring with more guaranties (Boratyński et al., 2010). However, an elevated BMR could be counterproductive in resource-poor habitats, so that the selected BMR must reach a compromise with the nutritional characteristics of the territory. Our study reveals differences in RMR values of females between bioclimatic ranges which may reflect an uneven availability of food resources along the year. Mueller and Diamond (2001) pointed out that animals from variable environments might have evolved to lower MR to ensure the metabolism in less productive periods. Hence, the environmental variability in mesomediterranean subpopulations could explain the lower RMR of these females. On the other hand, there are no significant differences in the RMR of males; the value of the supramediterranean-individuals is slightly higher than for the mesomediterranean. The energetic inversion in males does not require an additional level with which face breeding costs, so they have less RMR than females for the same habitat conditions.

According to Hayes (1989) and Willmer et al. (2005), BMR is intrinsically joined to the maximal aerobic metabolic rate or maximum rate of oxygen consumption. This latter rate represents the maximum physical activity or maximum thermoregulatory cost that an individual can support (Weibel et al., 2004). In this sense, a priori, a high BMR could be advantageous because it could pose a high level of available energy to escape from depredators or survive under cold temperatures, however it also could imply disadvantageous effects as consequence of the food search to maintain that higher energetic level (e.g. increase in the exposition to depredators) (Brown, 1988; Lovegrove, 2000). The RMR of *I. cabrerae* is the compromise reached by the species between the nutritional quality of their habitats and their activity habits, maybe a higher RMR could favour Cabrera voles but their environments would not be able to keep it.

In short, the study of the metabolic behaviour from an intraspecific approach provides a more realistic knowledge of the response of the species to an environmental gradient in contrast to interspecific studies which approximate the species' responses with average trends. Our intraspecific approach allowed us to identify differences in metabolic rates with changes in habitat conditions, mainly associated to thermal environments. Thus, thermoregulatory mechanisms seems to be locally adapted to bioclimatic zones, so that under warmer conditions the subpopulations' strategy was a later beginning of TNZ and higher Cm, and vice versa. Therefore under a framework of the global change, detecting the physiological assembling of the species to the environment can provide new insides to design effective protection programmes to improve the conservation status of an endemic species as Cabrera voles.

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