Original Investigation

Acclimating to thermal changes: Intraspecific variation in a small mammal from the Andes Mountains

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ABSTRACT

Ambient temperature strongly affects an ecosystem's characteristics as well as the attributes of individuals, eventually determining the distribution of populations and species. Phenotypic plasticity plays a central role in the administration of energy under thermal variation through traits underlying energy acquisition and expenditure. A powerful approach to assess acclimation ability to environmental variation is studying relevant traits along natural geographic gradients. Our goal was to assess and quantify in the small rodent *Phyllotis xanthopygus*, changes in traits relevant to energy balance in response to its thermal landscape. We compared energy intake and digestibility by animals from sites at different elevations under different temperatures in the laboratory. Results showed an increase in energy acquisition rates by the lower-elevation individuals to cope with low temperatures, while high-elevation animals appeared unaffected by this treatment. After acclimating to warmer conditions, all individuals showed a similar decrease in energy intake, irrespective of their origin site. We also assessed thermal conductance in individuals from different elevations and found that animals from higher sites exhibited lower heat loss rates. Our evidence suggests that heat conservation differences could in part account for differences among high and low elevation animals in the ability to cope with low temperatures. The lack of plasticity under the warm thermal treatment conforms to recent reports of high conservatism on the upper limit of the thermoneutral zone. *P. xanthopygus* displays intraspecific variation in the response to temperature, and we propose that this is highly relevant to model its chances in a warming environment.

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Introduction

Ambient temperature is a major determinant of an organism's life history traits. It influences resource availability, morphology, physiology, and behavior of individuals, eventually determining the distribution of populations and species. In animal species, this is reflected in the energy balance and thermal range achieved within a given habitat (Pörtner et al., 2006). Studying the variation of physiological traits along environmental gradients (Piersma and Lindström, 1997) allows the examination of a species overall thermal tolerance and its acclimation ability (Hammond et al., 2001; Chown et al., 2004). Understanding the physiological aptitude of species is crucial for developing sound biodiversity conservation strategies in the face of rapid climate change (Arlettaz et al., 2000).

Phenotypic plasticity is the ability of a species to produce different phenotypes under different environmental and trophic conditions (Pigliucci, 1996). Preferred traits to evaluate organisms' plastic responses should be intrinsically variable and relevant to environmental factors, such as temperature is relevant to energy balance (Bradshaw, 2003). Thus, phenotypic plasticity plays a central role in the administration of energy under environmental variation, through characters related to the acquisition and expenditure of energy. An elevation gradient represents an appropriate context for the assessment of plastic responses in energy balance relevant traits since it encompasses conspicuous thermal changes across relatively short distances.

Over the last years, ecological and evolutionary physiology has emphasized the importance of studying intraspecific variation as a substrate for natural selection (McNab, 2002). *Phyllotis xanthopygus* is a good animal model to accomplish this because it has a widespread geographic distribution in South America, which covers a steep elevation range in the Andes Mountains as well as a broad climatic cline (Steppan et al., 2007). The genus *Phyllotis* has been thoroughly studied under this approach showing both phenotypic plasticity and genetic variability. Main findings include a

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genetic basis for some traits linked to energy balance (Nespolo et al., 2003), a relationship between metabolic rate and habitat productivity (Bozinovic et al., 2007), an effect of ambient temperature and energy demands on digestive functions (Naya et al., 2005), and responses to changes in ambient temperature and diet (Bozinovic and Nespolo, 1997; Rezende and Bozinovic, 2001). Particularly for *P. xanthopygus*, phenotypic plasticity plays an important role in cold acclimation through changes in thermogenic capability (Nespolo et al., 1999). This is favorable in seasonal environments, where the species reduces energy expenditure in warmer periods (Stearns, 1989), and could also explain its wide range of distribution.

Heat generation ability and acclimation temperature are negatively correlated (Nespolo and Rosenmann, 1997). Likewise, heat conservation ability (i.e. thermal conductance) would be influenced by the thermal landscape. According to Sibly (1981) the digestive strategy maximizing net energy acquisition rate will be favored by natural selection. Thus, the interplay between food intake, digestive processing capacity and energy loss is expected to be coupled with demands. Based on this, here we analyzed how diverse experimental temperatures affect energy balance related traits in individuals of *P. xanthopygus* collected at different sites along an elevation cline. We hypothesize that animals adjust energy intake and energy digestibility to meet their requirements; thus we expect that before acclimation to the laboratory, individuals captured in higher elevations will show higher energy intake and assimilation of digestible energy, than individuals from lower elevations. On the other hand, acclimating to a particular environment (usually thermal) provides an organism with advantages within that environment (Leroi et al., 1994). Therefore, we hypothesize that subsequent thermal regimes in the laboratory will lead to changes in the cited energy acquisition traits. More specifically, individuals will increase rates of energy intake and assimilation under cold experimental conditions, compared to warm thermal treatments. Nevertheless, animals from different elevations could respond differently in spite of uniform experimental conditions, due to ontogenetic-based traits raised under diverse thermal regimes in the field. Regarding thermal conductance, we expect, under the same theoretical framework, that animals from higher elevations will show lower heat loss rates than lower elevation ones. A better understanding of a species performance and responses along thermal gradients enables modeling its chances of persistence, migration or resilience to climate change.

Material and methods

Study area

The study area is located at El Manzano Histórico Reserve, Mendoza Province, Argentina. We established sampling sites at 1700, 2300 and 3100 m a.s.l. along an elevation transect. Sites were 3 to 4 km apart, encompassing gradual changes in the landscape. According to Méndez (2011), these sites belong to different phytogeographic regions: sites at 1700 and 2300 m a.s.l. correspond to the Andina ecoregion and at 3100 m a.s.l. to the Alto Andina ecoregion.

In order to characterize the environmental temperature range we set up three dataloggers – Extech® USB Temperature Dataloggers, range −40 to 70 °C, resolution 0.1 °C, accuracy ± 1.0 °C (−10 to 40 °C) –, one at each elevation site, recording temperatures at 3 h intervals for a period of one year (13 January 2013 to 06 Febrary 2014). Each datalogger was previously calibrated in the laboratory and set at ground level (beneath the rocks, within cracks possibly used by the mice) in the field.

Animals and acclimation

In order to test for geographic related variability, a Common Garden Experiment (Garland and Adolph, 1991) was performed,

comparing individuals from different sites acclimated to common experimental situations in the lab. Thirty non reproductive adult *P. xanthopygus xanthophygus* (10 from each site) were captured using Sherman traps (Möller® $8 \times 9 \times 24\,cm^3$) between February and March 2013. All animals belonged to the same subspecies, with a sex ratio of 1/1. Genetic analyses on the individuals showed no genetic differentiation and high gene flow levels among animals from all sites (Ojeda et al., 2013).

In the laboratory, animals were maintained on chinchilla food pellets and water *ad libitum*. Individuals were housed individually in $30 \times 30 \times 40\,cm^3$ size cages and maintained on 12L: 12D photoperiod and at 25 °C ambient temperature (standard laboratory conditions—Bozinovic and Nespolo, 1997; Nespolo et al., 1999).

To estimate Thermal Conductance, 15 non reproductive adult individuals (five from each site) were collected between February and March 2013, and housed individually under maintenance conditions until the trial.

Experiments

The first experiment consisted of measuring energy intake, digestibility and weight on each individual subsequently exposed to four treatments. The first measurement was carried out immediately after the animals' capture at 25 °C (treatment I), in order to detect the residual effect of distinct thermal conditions endured by them in the field. Subsequently, individuals were acclimated during 8 weeks to 25 °C, 16 °C and 31 °C; treatments two (II), three (III) and four (IV) respectively. Energy intake, digestibility and body weight were measured after the acclimation period to each temperature in order to assess the effect of thermal treatments on animals from distinct elevations.

Experimental temperatures were chosen considering two elements. First, initial field estimates evidenced an ambient temperature differential of 12 °C between the most extreme sites along the elevation cline considered. In fact, mean ambient temperatures during the month of animal captures were 24.29 ± 4.14 °C (1700 m a.s.l), 18.96 ± 2.33 °C (2300 m a.s.l), and 12.73 ± 2.34 °C (3100 m a.s.l), which was used as preliminary information on the thermal range in the field. Finally, baseline studies on *Phyllotis*, searching for thermal related responses have acclimated animals at 15 and 30 °C, these alternative temperatures corresponding to relatively high and low energy demanding conditions, respectively, with typical laboratory maintenance temperatures at 25 °C (Bozinovic and Nespolo, 1997; Nespolo et al., 1999; Canals et al., 2009).

Measurements of energy intake and energy digestibility from food (Chindiet®) were performed over the course of four days, which is a standard period for obtaining representative estimates with sufficient resolution (Sales and Janssens, 2003, and references therein). Each day, samples of leftover food and feces were dried at 60 °C in a dry heat sterilizer for a week and weighted in a precision balance (resolution = 0.01 g). Food intake was measured as the difference between the amount of dried food offered and left per day. The energy content per gram in feces and leftover food was measured using a Parr Bomb Calorimeter which was multiplied by the dry weight of samples. Energy intake which was calculated per day for each individual under each treatment according to Qi–Qe, where Qi is the daily rate of energy in food intake and Qe is the daily rate of energy wasted in feces. Digestibility is the extraction efficiency of energy, dry matter or other nutrients from food, calculated as [(Qi–Qe)/Qi] × 100%. This method underestimates digestive efficiency because it includes the contribution of metabolic wastes, non-reabsorbed secretions of the digestive system and microorganisms (Naya and Bozinovic, 2006). Body weight was measured at the beginning of each four-day trial to detect differences from the acclimation thermal conditions.

To perform the thermal conductance experiment, heat loss rates were assessed using the method of Morrison and Tietz (1957). Animals were euthanized and experiment was performed in compliance with the legislation for the protection of animals used for scientific purposes: http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm. Using a digital thermometer with dual display backlight, we recorded the change in colonic body temperature (T_b) of sacrificed animals previously warmed to 37 °C, in a chamber at a temperature (T_a) of 5 ± 2 °C. At 3 min intervals we recorded T_b and T_a until a thermal differential of 10 to 15 °C was achieved. Thermal conductance was calculated for each individual as proposed by Bozinovic et al. (1987): Thermal conductance = b x Hm ($^{cal}/_g$ h °C), where b = slope of the curve ln ($T_b - T_a$) vs. Time (min) and Hm = specific heat of a mammal (0.84 $^{cal}/_g$ °C).

Data analysis

Differences between the annual mean ambient temperatures among the three elevation sites were estimated using a non parametric analysis (Kruskall Wallis and post-hoc test) since residuals of the relationship between temperature and elevations were not consistent with normal distribution assumptions. Temperature means were also calculated by season in order to show the intra-site annual variation.

Data from days one to four were averaged to obtain a representative value per individual under each treatment. We performed a multivariate analysis of variance (MANOVA) to analyze data from treatment I (25 °C I). We compared energy intake, energy digestibility and body weight among groups from different elevations before acclimation to laboratory conditions. Afterwards, we calculated differences from the pre acclimation conditions with data on energy intake, energy digestibility and body weight, obtained after acclimation to the subsequent temperatures (25 °C, 16 °C and 31 °C). We used these differences as response variables to test for the effect of elevation and experimental treatment. We separately analyzed variation in energy intake, energy digestibility and body weight with Factorial ANOVAs, using elevation (1700, 2300 and 3100 m a.s.l) and experimental treatment (25 °C II, 16 °C and 31 °C) as fixed factors.
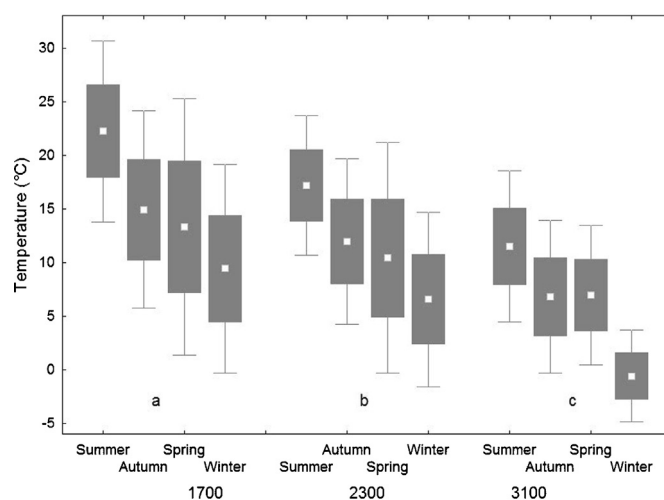
Thermal conductance among individuals from the different sites was statistically analyzed with an analysis of covariance (ANCOVA), with heat loss rate as the response variable, elevation as fixed factor, and body weight as a covariable. All statistical analyses were performed using the Statistica (StatSoft., 2007) package for Windows.

Results

There was a marked decrease in annual mean ambient temperature towards higher elevations: 14.91 ± 6.83 °C (1700 m a.s.l.), 11.15 ± 5.65 °C (2300 m a.s.l) and 7.40 ± 5.6 °C (3100 m a.s.l.). Mean temperatures showed significant differences among sampling sites ($H(2) = 2524.97$, $p < 0.05$), despite a large seasonal variation (Fig. 1).

In treatment I −25 °C immediately after capture—the general effect of elevation site on the response variables was not significant (Wilks lambda = 0.82, $F(6, 50) = 0.84$, $p = 0.54$). Body weight and energy digestibility were unaffected by origin site. Nevertheless, animals from 3100 m a.s.l. showed significantly higher energy intake than those from 1700 m a.s.l., while energy intake by 2300 m a.s.l. animals was intermediate (Table 1, LSD post hoc test $p < 0.05$).
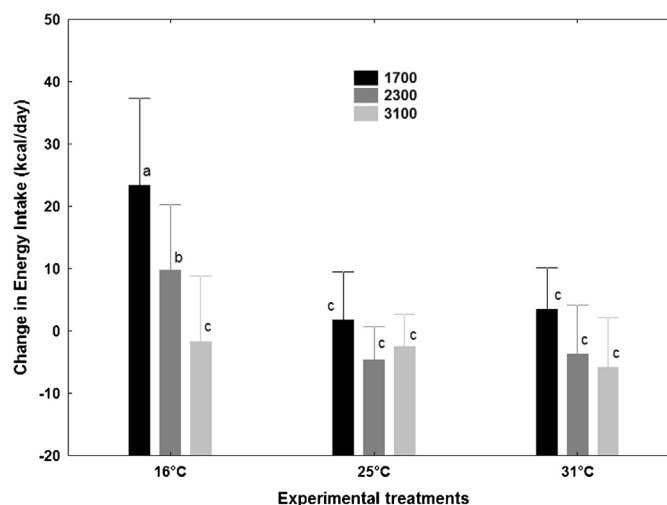
Throughout acclimation to laboratory, energy intake changed significantly, showing treatment ($F(2, 79) = 10.12$, $p < 0.01$) and elevation effects ($F(2, 79) = 8.67$, $p = <0.01$). There was no statistical effect of the treatment-by-elevation interaction ($F(4, 79) = 2.01$,

Fig. 1. Mean ($\pm$ SD and 95% CI) ambient temperature at the three study sites as measured during a complete year cycle (from 13 January 2013 to 06 Febrary 2014). Different letters indicate pairwise statistically significant differences ($p < 0.05$).

$p = 0.10$). After acclimation to 16 °C, we found significant differences among elevations with a pattern opposed to that under treatment I, i.e. animals from lower elevations showed higher energy intake than animals from higher sites (Fig. 2, LSD post hoc test $p < 0.05$). Eight weeks exposure to 25 °C resulted in no elevation pattern since animals from 3100 and 2300 m elevation decreased their energy intake with respect to pre acclimation conditions (Fig. 2, LSD post hoc test, $p < 0.05$). Finally, after acclimation to 31 °C, energy intake was close to initial levels for first group (1700 m a.s.l.), and lower for the other ones (2300 and 3100 m a.s.l.), without statistical differences among them (Fig. 2, LSD post hoc test $p < 0.05$).
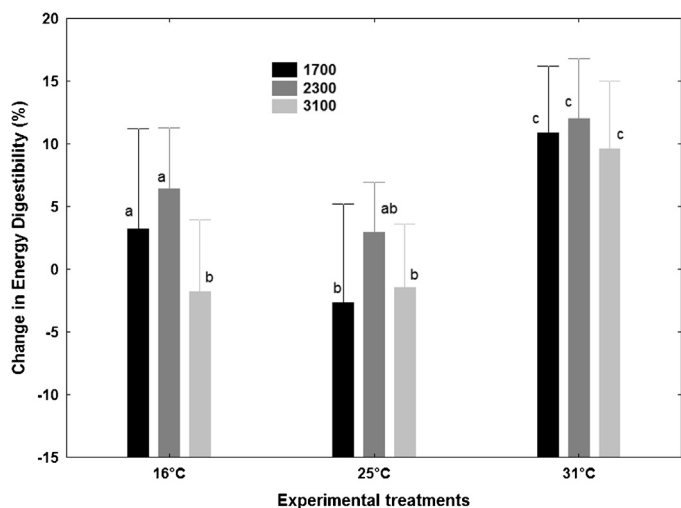
Energy digestibility varied respect to pre acclimation values and this change was mainly due to the effect of thermal treatments ($F(2, 79) = 14.85$, $p < 0.01$). There were no significant effects of elevation ($F(2, 79) = 2.83$, $p = 0.06$) or elevation-by-experiment interaction ($F(4, 79) = 0.58$, $p = 0.67$). However, a pattern emerged at 16 °C, with animals from the two lower elevation sites showing increases in digestibility means compared to those from 3100 m a.s.l. which showed no changes with respect to the pre acclimation conditions (Fig. 3, LSD post hoc test $p < 0.05$). Finally, mean

Fig. 2. Changes in energy intake under experimental temperatures (16 °C, 25 °C, 31 °C) by animals stemming from different elevation levels (1700, 2300, and 3100 m a.s.l.). Values presented are weighed means and vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences among means ($p < 0.05$).

Table 1
Mean energy intake (M ± SE in kcal/day), mean energy digestibility (M ± SE in %), and mean body weight (M ± SE in g) for animals from each elevation (1700, 2300, and 3100 m a.s.l.). MANOVA results for treatment 1 (comparison of elevation groups on baseline pre acclimation conditions at 25 °C I). Means with different letters indicate statistically significant differences (p < 0.05).

Elevation (m a.s.l.)	Energy intake (kcal/day)	Energy digestibility (%)	Body weight (g)
1700	32.83 ± 2.65[a]	67.87 ± 2.39[a]	59 ± 3.76[a]
2300	37.80 ± 3.11[ab]	64.76 ± 1.63[a]	60.3 ± 5.54[a]
3100	41.34 ± 2.97[b]	67.34 ± 2.66[a]	64.3 ± 3.94[a]

Fig. 3. Changes in energy digestibility under experimental temperatures (16 °C, 25 °C, 31 °C) by animals stemming from different elevation levels (1700, 2300, and 3100 m a.s.l.). Values presented are weighed means and vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences among means (p < 0.05).

digestibility increased substantially in all elevation groups after acclimation to 31 °C (Fig. 3, LSD post hoc test p < 0.05).

Body weight showed increases in all elevation groups with acclimation to experimental conditions. Thermal treatments had a statistically significant effect on body weight change ($F(2, 79) = 68.28$, $p < 0.01$). There were no significant effects of elevation ($F(2, 79) = 0.66$, $p = 0.51$) or elevation-by-experiment interaction ($F(4, 79) = 0.16$, $p = 0.95$). It is noteworthy that while during treatments II and III (16 °C and 25 °C), body weight slightly changed, after acclimation to 31 °C temperature, all animals significantly gained weight irrespective of their site of origin (Fig. 4, LSD post hoc test $p < 0.05$).

Thermal conductance varied significantly across sites ($F(2, 11) = 3.99$, $p = 0.049$) with animals from lower elevations showing higher heat loss rates than animals from higher ones (Table 2). Body weight significantly covaried with heat loss rate ($F(1, 11) = 9.38$, $p = 0.01$).

Table 2
ANCOVA results for thermal conductance. Comparison of heat loss rates among animals from each altitude (1700, 2300, and 3100 m a.s.l.). Values are means and standard errors. Means with different letters are significantly different (LSD post hoc test p < 0.05).
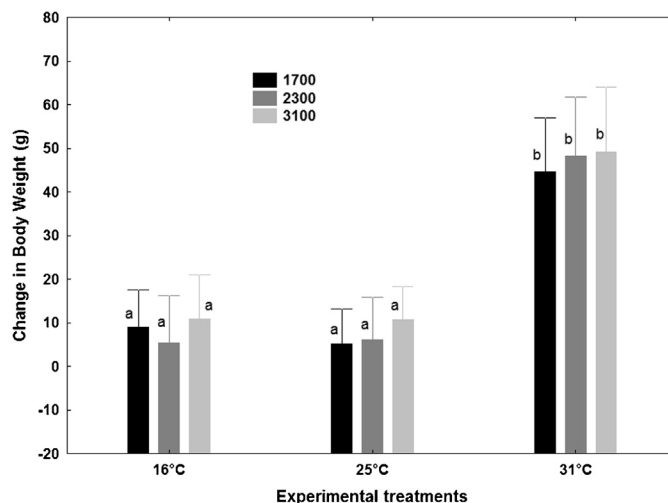
Thermal conductance (cal/g h °C)

Altitude	Mean	Standard error	N	LSD test
1700	0.76	0.06	5	a
2300	0.69	0.06	5	ab
3100	0.52	0.06	5	b

Discussion

The ability to cope with environmental contingencies is promoted by natural selection (McNamara and Houston, 1996). Therefore, studying the plasticity of phenotypic responses to variable environmental conditions provides good insights into species' chances of adapting to change. Our study confirms that *P. xanthopygus* exhibits variation in energy balance related traits probably coupled with changes in the thermal landscape with elevation. These results will be discussed along two main perspectives: (1) the mechanisms used to cope with a variable environment, referring to the responses observed, and the potential implications of the species' ecology and evolutionary history; (2) the pattern of intraspecific variability, drawing possible lessons for understanding its implications in a climate change scenario.

Mechanism

P. xanthopygus displayed some intraspecific variation in energy intake at the onset of acclimation to laboratory conditions, which we believe mirrors natural species' exposure to distinct environmental circumstances, namely to rather different thermal ranges. After 8 weeks acclimating to 25 °C, the previous difference in energy intake between highest and lowest elevation animals disappeared. This is consistent with our expectations, and concurs with findings by Hayes and O'Connor (1999) who reported in *Peromyscus maniculatus* high energy demands at high elevations and a loss of intraspecific variation after exposure to warm temperatures. In *Phyllotis darwini*, our model's sister species, responses to low temperatures comprise increases in energy intake, aerobic scope and size of intestines, proliferation of brown adipose tissue and increases in metabolism, among other highly correlated traits

Fig. 4. Changes in body weight under experimental temperatures (16 °C, 25 °C, 31 °C) by animals stemming from different elevation levels (1700, 2300, and 3100 m a.s.l.). Values presented are weighed means and vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences among means (p < 0.05).

(Nespolo et al., 1999, 2003; Naya et al., 2005). A high thermogenic capacity needs sophisticated, energetically expensive physiological machinery that is modulated by environmental signals like photoperiod or ambient temperature (Stearns, 1989). Its plasticity is considered to allow small non-hibernating eutherian mammals to cope with seasonal variability (Bacigalupe et al., 2004). For *P. xanthopygus*, Nespolo et al. (1999) reported phenotypic plasticity in metabolic indices due to acclimation to low temperatures, but no variation at 25 °C. Our spatial scale differences are possibly due to some of the cited mechanisms underlying animals' acclimatization to different elevations in the field. Plasticity of these traits explains the loss of initial intraspecific differences after acclimation to 25 °C.

Experimental exposure of animals to 16 °C and subsequent measurement of response variables revealed a new intraspecific pattern. Our prediction, since differences disappeared under laboratory maintenance conditions (25 °C), was that animals would increase their energy intake and/or digestibility under 16 °C irrespective of their origin elevation. Nevertheless, we found elevation-specific adjustments to cope with low temperatures. In fact, individuals from higher sites appeared to be unaffected by this thermal treatment, whereas lower-sites' ones responded by improving their energy acquisition, with a pattern inversely correlated with elevation. These results are concurrent with previous paired comparisons of lowland and highland populations (Hammond et al., 2001).

Correlation between intake and energy demands is usual in small mammals (Hammond and Diamond, 1992; Hammond et al., 2001), and our findings conform to a theoretically expected rise in energy acquisition rate (Hayes and Chappell, 1986; Bozinovic and Nespolo, 1997; Rezende et al., 2004). Nevertheless, the occurrence and intensity of this change show intraspecific variations, with lower-elevation animals needing more energy to deal with the low temperature treatment. This is also supported by thermal conductance data, suggesting that energy acquisition is related to heat-losses, as proposed by McNab (2002).

In fact, our results for thermal conductance show site-related differences, with higher heat loss rates for lower elevation animals. Thermal conductance usually correlates positively with environmental temperature and inversely with body size (Brown and Lee, 1969). This trait could compensate for heat loss when the temperature differential between body and environment increases, for instance along our elevation cline. Since we found no body size variation with respect to elevation, fur thickness is a likely response to cold. As a result, high-elevation individuals could more efficiently undergo low ambient temperatures due to a relatively higher insulation. Persistent differences in thermal conductance through different acclimation treatments are corresponding with year-round thermal ranges recorded at each elevation level (Fig. 1).

The final treatment at 31 °C yielded the most noticeable differences in the response variables among treatments. Energy intake slightly decreased while digestibility increased substantially with no elevation-related differences. Since questions have been traditionally guided to understand how animals deal with challenges to energy balance (i.e. low temperatures), fewer studies have tested physiological traits related to abundant energy resources (i.e. high temperatures, see Speakman and Król, 2010 for a discussion on this subject). Among them, responses to warm experimental treatments include: lowered energy intake with no elevation-related differences (Hammond et al., 2001), increase in body weight and decrease in maximum and resting metabolic rate (Novoa et al., 2005; Nespolo and Rosenmann, 1997; Nespolo et al., 1999, 2001; Antinuchi and Bush, 2000). As for *Phyllotis*, there is evidence of decrease in energy intake (Bozinovic and Nespolo, 1997), slowdown of digestion turnover time (Naya et al., 2005) and depressed thermogenic capacity when acclimated to 30 °C (Nespolo et al.,

2001). Our results under this thermal treatment are strongly consistent with a decrease in energy expenditure, a slowdown in passage and digestive rates, a relatively small amount of feces produced and therefore an increase in the proportion of energy extracted from food. It is also supported by the concomitant increase in body weight. These responses make sense since the reported thermoneutral zone for the species is between 28 °C and 36 °C (Bozinovic and Rosenmann, 1988). Thus, at 31 °C animals are not thermoregulating and their energy requirements decrease. This is probably common to all individuals, and explains the lack of intraspecific variation in digestive traits under this treatment.

Pattern

The evidence corroborates that our model species is plastic in the physiological traits considered, and that this plasticity occurs at two levels: among elevation groups and within them. This study assessed via a common garden experimental design, geographically based differences in physiological traits, since environmental effects could promote irreversible developmental plasticity (Garland and Adolph, 1991). Moreover, costs of plasticity could imply certain level of phenotypic differentiation among groups exposed throughout their life to a given environmental range. As expected, thermal ranges across our elevation gradient were markedly distinct in spite of seasonal variation, which possibly represents sustained site-specific conditions. We also found a broad repertoire of responses to preacclimation field conditions and low temperature experimental treatment, as well as a more uniform, and yet different response after acclimation to warmer conditions. This is consistent with the reported ability to fine-tune thermogenic capacity, related by Nespolo and Rosenmann (1997) to the thermal history of the species. *P. xanthopygus* origin and diversification occurred mostly in mountain environments (Steppan et al., 2007), therefore low temperature acclimation ability would have been favored by natural selection (Nespolo et al., 1999). In fact, an among-species comparison by Rezende et al. (2001) indicates that the mechanisms related to metabolic plasticity in response to low temperatures remain conserved in the genus *Phyllotis*. As for our results, differences in the response to the cold thermal treatment among high and low elevation animals suggest intraspecific variation in the lower limit of the species' thermoneutral zone. Regarding the response to the warm thermal treatment, the lack of intraspecific variability conforms to recent reports of high conservatism in the tolerance to heat (Araújo et al., 2013). Future research should approach the versatility of this cold-adapted species to deal with a warming environment.

Highlands all over the world are considered especially vulnerable to climate change, with predictions of temperature increase being twice higher than for lowland areas. And the Andes are no exception: forecasts indicate a 2–4 °C increase over the next 100 years (Labraga and Villalba, 2009). Predicting future geographic distribution and the survival chance of biodiversity requires refined climate-based envelope models that incorporate precise information about key eco-physiological attributes. In this sense, information on relevant traits should include their intrinsic variability, since mechanisms underlying the response to thermal conditions vary across species' distributions as this study demonstrates along an elevation cline.

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