

RESEARCH ARTICLE

Is there metabolic cold adaptation in terrestrial ectotherms? Exploring latitudinal compensation in the invasive snail *Cornu aspersum*

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ABSTRACT

Lower temperatures, extreme seasonality and shorter growing seasons at higher latitudes are expected to cause a decline in metabolic rates and annual growth rates of ectotherms. If a reduction in the rates of these biological processes involves a reduction in fitness, then organisms may evolve compensatory responses for the constraints imposed by high-latitude habitats. To test the existence of a latitudinal compensation in ectotherms, we used a common-garden experiment to investigate the extent to which the level of energy turnover (measured as standard metabolic rate, SMR) and the energy budget (energy allocation to growth) are affected by climatic constraints in three populations of the land snail *Cornu aspersum*, distributed across a latitudinal gradient of 1300 km in Chile. Our results did not support the existence of a latitudinal compensation in metabolic rates (metabolic cold adaptation). However, there was a countergradient variation (CnGV) for growth rate in which the highest latitudinal population exhibited greater growth rates than their counterparts from lower latitudes. Surprisingly, this CnGV pattern was accompanied by a lower apparent dry-matter digestibility, which could highlight a differential assimilation of ingested nutrients into somatic tissue, revealing enhanced growth efficiency in snails from the highest latitudinal habitat. Our evidence highlights that adjustments in energy allocation to the digestive machinery and to protein storage could act as a latitudinal compensation for enhanced growth efficiency in snails from the highest latitudinal population.

KEY WORDS: Metabolic rate, Countergradient variation, Energy allocation, Growth

INTRODUCTION

Environmental variation across geographical gradients is a key driver in the evolution of biological diversity, giving rise to genetic and phenotypic variation within species (Culumber et al., 2012), and affecting survival and reproduction of organisms (Gaston, 2009; Gunderson and Leal, 2012). Such variation has been well documented across latitudinal gradients, in which climatic conditions, season lengths, ambient temperatures and resource availability are paramount factors affecting the rate of most biological processes (Somero, 1995; Huey and Berrigan, 2001; Kingsolver and Huey, 2008; Hochachka and Somero, 2002; Lindgren and Laurila, 2009; Pörtner et al., 2005). For example,

lower mean temperatures, extreme seasonality and shorter growing seasons at higher latitudes are expected to cause a decline in metabolic rates (Jose et al., 2009) and annual growth rates of ectotherms (Yamahira and Conover, 2002). Nevertheless, if a reduction in the rates of these biological processes involves a reduction in fitness, then organisms may evolve compensatory responses for the constraints imposed by high-latitude habitats (Yamahira and Conover, 2002; Levinton, 1983; Fanguie et al., 2009).

In principle, two alternative models could explain the evolution of latitudinal compensation in ectotherms. One model is local thermal adaptation, which predicts that organisms from high latitudes should function better at low temperatures (i.e. shift of the thermal optimum) in relation to their counterparts from lower latitudes (Tattersall et al., 2012; Angilletta, 2009; Gaitán-Espitia et al., 2013a). An alternative model of latitudinal compensation focuses on latitudinal differences in the length of the growing season rather than the local mean temperature (Conover and Present, 1990). This model is based on the countergradient variation (CnGV), which occurs when genetic differences counteract environmental effects, reducing the phenotypic differentiation between populations along an environmental gradient (Conover and Schultz, 1995; Niewiarowski and Angilletta, 2008). The CnGV model predicts that organisms compensate for the climatic constraints at high latitudes by increasing performance, growth and fecundity relative to their lower latitude counterparts when they are reared and compared at the same temperature (Levins, 1969; Conover and Schultz, 1995; Schultz et al., 1996; Fanguie et al., 2009; Yamahira and Conover, 2002). This compensation allows the completion of the juvenile stages during the short growing season and enhances overwinter survival under harsh conditions (Lindgren and Laurila, 2009).

These models of latitudinal compensation are not mutually exclusive. In fact, as temperature and length of growing season covary across latitudinal gradients, organisms may adopt a mixed strategy by adapting to differences in both temperature and seasonality (Conover and Present, 1990; Yamahira and Conover, 2002). Nevertheless, observations from eco-physiological studies have shown that adaptive responses to climatic constraints at high latitudes usually follow the CnGV model (Yamahira and Conover, 2002). Latitudinal compensation usually comprises a rise in mitochondrial ATP synthesis capacity (Sommer and Pörtner, 2002; Sommer and Pörtner, 2004), and in metabolic rates of organisms at high latitudes (Clarke, 1993; Pörtner et al., 2005; Conover and Schultz, 1995). Increased metabolic rates are predicted to be an adaptive response, allowing accelerated physiological processes in environments that feature shorter periods of optimal conditions (Addo-Bediako et al., 2002; Clarke, 1993; Clarke, 2001), and should effectively counteract the environmental effect of depressed temperatures on growth rates (Conover and Schultz, 1995; Chown and Gaston, 1999). This example of CnGV has been termed

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metabolic cold adaptation (MCA) (Clarke, 1993; Clarke, 2001; Schaefer and Walters, 2010; White et al., 2012).

In general, MCA has been the subject of great controversy because whereas some studies support the MCA hypothesis (Addo-Bediako et al., 2002; Gaston et al., 2009; White et al., 2012; Hodkinson, 2003), other studies have failed to find an increase in O_2 consumption at high latitudes (Lardies et al., 2004a; Jordan et al., 2001; Steffensen, 2002; Steffensen et al., 1994). Additionally, much of the controversy surrounding MCA has centred on methodological issues that could confound the outcome of these studies (Chown et al., 2003; Bozinovic et al., 2011). The most straightforward approach to test for a CnGV pattern such as MCA is the comparison of populations at a single test temperature (Schaefer and Walters, 2010) using a common-garden experiment. This approach permits elimination of the possibility that differences between groups are due to maternal effects (i.e. modifications of offspring phenotype caused by the environment provided by the mother) (Mousseau and Fox, 1998), other pre-fertilization environmental influences (Suarez-Alvarez et al., 2012) or acclimation (i.e. phenotypic flexibility) (Piersma and Drent, 2003).

Using a common-garden experiment, we examined the MCA hypothesis in ectotherms at the intra-specific level by testing for the existence of latitudinal compensation in the land snail *Cornu aspersum* (O. F. Müller 1774). This land snail is characterized by physiological and morphological adaptations (e.g. metabolic depression, supercooling ability, reduction of the rate of water loss, thicker shell and epiphragm) that enable it to inhabit a great variety of terrestrial environments (Vorhaben et al., 1984; Bishop and Brand, 2000; Machin, 1967; Gaitán-Espitia et al., 2013c). Here, we analysed the extent to which the level of energy turnover (measured as standard metabolic rate, SMR) and the energy budget (energy allocation to growth) are affected by climatic constraints in three populations distributed across a latitudinal gradient of 1300 km in Chile.

RESULTS

Population growth rate

Populations of *C. aspersum* across the latitudinal gradient (La Serena 29°S; Constitución 35°S; Valdivia 29°S) exhibited similar parameters (Table 1) and patterns of growth curves (Fig. 1A). In the

three populations, values of specific growth during the first month of age were the largest (Table 1). However, the parameters estimated for monthly growth rate with the Gompertz equation showed differences between populations (maximum likelihood test, $P < 0.05$) characterized by an increasing pattern with age in the La Serena and Valdivia populations, but not in Constitución (Table 1). This difference is explained by the greater rate of growth exhibited by snails from the Constitución population at 4 months of age (Table 1 and Fig. 1A; one-way ANOVA, $F_{2,1451}=13.01$, $P < 0.001$). However, the overall growth rate showed the greatest values in the Valdivia population (one-way ANOVA, $F_{2,1451}=8.01$, $P < 0.001$; Fig. 1B), producing greater body mass (M_b) in pre-adult snails from Valdivia compared with the other two populations after 6 months under the same rearing conditions (one-way ANOVA, M_b $F_{2,1451}=10.83$, $P < 0.001$). Moreover, analysis at early stages (i.e. eggs and hatchlings) revealed that the mass of the eggs (Table 2) differed among populations (one-way ANOVA, $F_{2,1451}=9.0$, $P < 0.001$), with larger eggs in La Serena and Valdivia populations compared with the Constitución population (Table 2; Tukey HSD test, $P < 0.05$). After eclosion, these differences were maintained in the mass of the hatchlings across the latitudinal gradient (one-way ANOVA, $F_{2,1451}=8.13$, $P < 0.001$) only because of the greater values for snails of the Valdivia population (Table 1; Tukey HSD test, $P < 0.05$).

Digestibility, energy expenditure and water balance

Of the physiological variables, only SMR was highly and significantly correlated with M_b ($r=0.71$, $P < 0.05$), whereas body dehydration was low but significantly correlated with apparent dry-matter digestibility ($r=0.10$, $P < 0.05$) and SMR ($r=0.08$, $P < 0.05$). Analysis of these variables among populations revealed a lack of differences in SMR (one-way ANCOVA; $F_{2,1450}=0.872$, $P=0.42$; Fig. 1C) and body dehydration (one-way ANOVA; $F_{2,1451}=1.24$, $P=0.29$). In contrast, dry-matter digestibility showed differences across the latitudinal gradient (one-way ANOVA; $F_{2,1451}=13.72$, $P < 0.05$), which was explained by lower values in snails from Valdivia (i.e. high latitude) compared with the other populations (Tukey HSD test, $P < 0.05$; Fig. 1D). Finally, after controlling for M_b , SMR and the other physiological variables were not correlated with the overall growth rate.

Table 1. Growth rate at different ages in populations of the land snail *Cornu aspersum*

Population	Age (months)	Body mass (g)	Lower 95% CL	Upper 95% CL	Growth rate (g month ⁻¹)	Specific growth (% month ⁻¹)
La Serena 29°S	1	0.07	0.02	0.12	0.04	68.82
	2	0.16	0.07	0.25	0.09	57.68
	3	0.25	0.09	0.41	0.09	35.88
	4	0.37	0.18	0.56	0.12	33.42
	5	0.56	0.30	0.82	0.18	33.51
	6	0.86	0.45	1.27	0.30	34.86
Constitución 35°S	1	0.08	0.02	0.13	0.07	77.3
	2	0.17	0.08	0.31	0.08	48.28
	3	0.23	0.12	0.42	0.05	24.31
	4	0.55	0.2	0.91	0.34	60.03
	5	0.65	0.29	1.01	0.08	12.83
	6	0.84	0.41	1.28	0.19	22.76
Valdivia 39°S	1	0.09	0.03	0.16	0.07	76.15
	2	0.17	0.09	0.27	0.07	42.89
	3	0.25	0.17	0.41	0.08	31.35
	4	0.41	0.18	0.63	0.16	38.57
	5	0.68	0.33	1.03	0.28	40.26
	6	0.92	0.52	1.31	0.25	25.45

Growth rate was measured as the increase in body mass (M_b) by a Gompertz equation: $L_t = L_\infty \exp[-G \exp(-gt)]$. L_t is the M_b at time t ; L_∞ is a constant that represents the asymptotic mass of the snails; $G = \ln(L_\infty/L_t)$; g is the growth rate constant (g month⁻¹); and t is time (age in months). CL, confidence limit. Maximum likelihood test, $P < 0.05$.

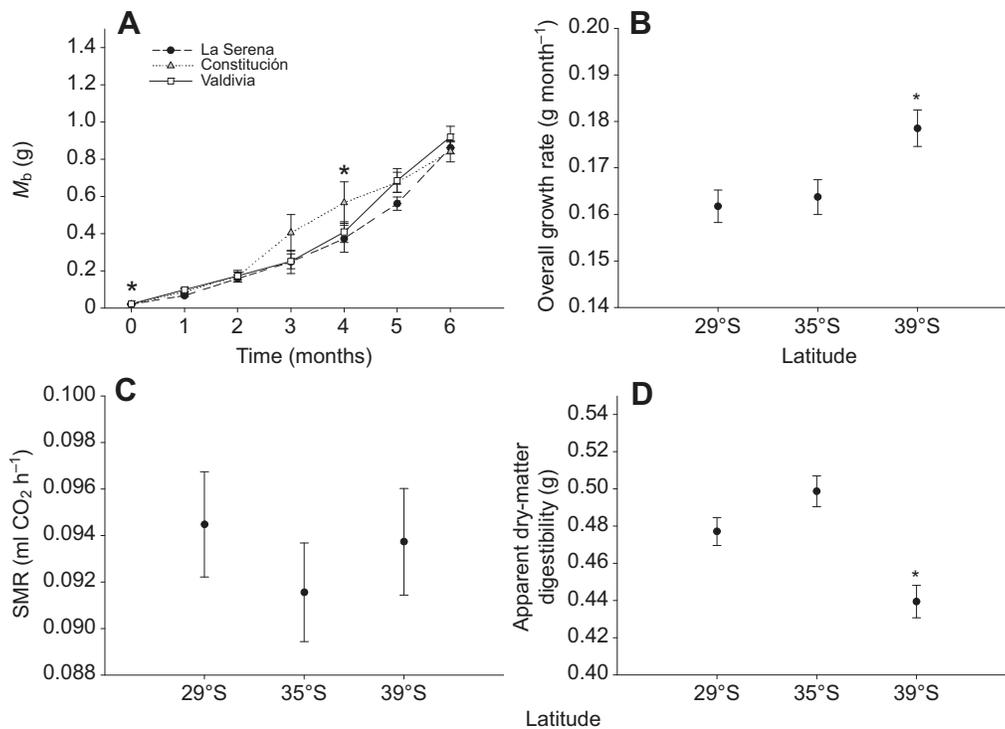


Fig. 1. Latitudinal variation in growth rate curves. (A) Body mass (M_b), (B) overall growth rate, (C) standard metabolic rate (SMR) and (D) apparent dry-matter digestibility. Circles and bars represent means and s.e.m., respectively. 29°S, La Serena; 35°S, Constitución; 39°S, Valdivia. Asterisks denote significant differences between populations ($P < 0.05$).

DISCUSSION

According to the hypothesis of MCA (Clarke, 1993; Wohlschlag, 1960), populations of ectotherms that inhabit colder environments should have increased metabolic rates to compensate for the negative effect of low temperatures and shorter growing season on growth rates (Conover and Schultz, 1995). This latitudinal compensation should allow faster growth in populations from high-latitude habitats relative to their counterparts from lower latitudes when compared at a common temperature (Levins, 1969; Conover and Schultz, 1995; Schultz et al., 1996; Álvarez et al., 2006; Yamahira and Conover, 2002). However, our results evidenced a lack of differences in the energetic costs of maintenance (measured as SMR) among populations of the land snail *C. aspersum* raised in a common-garden experiment after three generations. This could be the result of: (i) metabolic adjustments through phenotypic plasticity (Naya et al., 2011); (ii) the effect of common-garden conditions and acclimation in the laboratory (Gatten et al., 1988); or (iii) the minimization of climatic differences through a 'microhabitat effect', considered to be a behavioural adaptation to seek the optimum environment (e.g. orientation of the shell to facilitate heat flow or heat retention, climbing on to host plants to choose resting sites that are sheltered from harsh environmental conditions) (Hazel and Johnson, 1990). Nevertheless, the absence of differences in SMR could indicate that snails from the three populations required similar amounts of energy for maintenance during physical inactivity (Lindgren and Laurila, 2009) despite the differences in the climatic

constraints of their original environments (Gaitán-Espitia et al., 2013a).

Despite the lack of evidence in support of the predictions of the MCA (i.e. differences in SMR among populations of *C. aspersum*), our results revealed a pattern of CnGV in growth rates. In fact, snails from the highest latitude exhibited a greater overall growth rate than their counterparts from lower latitudes. This kind of countergradient response in growth rates has been found in other ectotherms at both intra- and inter-specific levels (Schultz et al., 1996; Conover and Present, 1990; Álvarez et al., 2006; Niewiarowski and Angilletta, 2008; Fangué et al., 2009; Conover and Schultz, 1995; Yamahira and Conover, 2002). Such a pattern suggests that the evolutionary response to short growing seasons and long winters at high latitudes favours genotypes with the capacity for rapid summer growth (Yamahira and Conover, 2002). However, it is important to recognize that CnGV models such as the MCA, rely on an energy budget context in which the energy allocation to growth is subject to constraints (e.g. the energy intake rate, digestion and absorption efficiency) and competing demands (e.g. maintenance, activity and reproduction) (Clarke, 1993; Weiner, 1992; Piersma and van Gils, 2011). Therefore, it could be expected that higher growth rates in populations from high latitudes involve some costs in other contributors to the energy budget (Chown and Gaston, 1999; Lindgren and Laurila, 2009; Pörtner et al., 2005; Conover and Present, 1990). Nevertheless, this was not found in our study, at least regarding maintenance metabolism.

Table 2. Physiological variables measured in three populations of the land snail *C. aspersum* across a latitudinal gradient

	Latitude	No. of snails	M_{eggs} (g)	SMR (ml CO ₂ h ⁻¹)	AMD (g)	BD (%)
La Serena	29°54'S, 71°15'W	495	0.0226±0.0003	0.0945±0.002	0.477±0.007	7.12±0.18
Constitución	35°20'S, 72°25'W	502	0.0201±0.0005	0.0916±0.002	0.498±0.008	6.91±0.19
Valdivia	39°38'S, 73°5'W	457	0.0235±0.0004	0.0937±0.002	0.439±0.008	7.21±0.22

M_{eggs} , egg mass; SMR, standard metabolic rate; AMD, apparent dry-matter digestibility; BD, body dehydration (means ± s.e.m.).

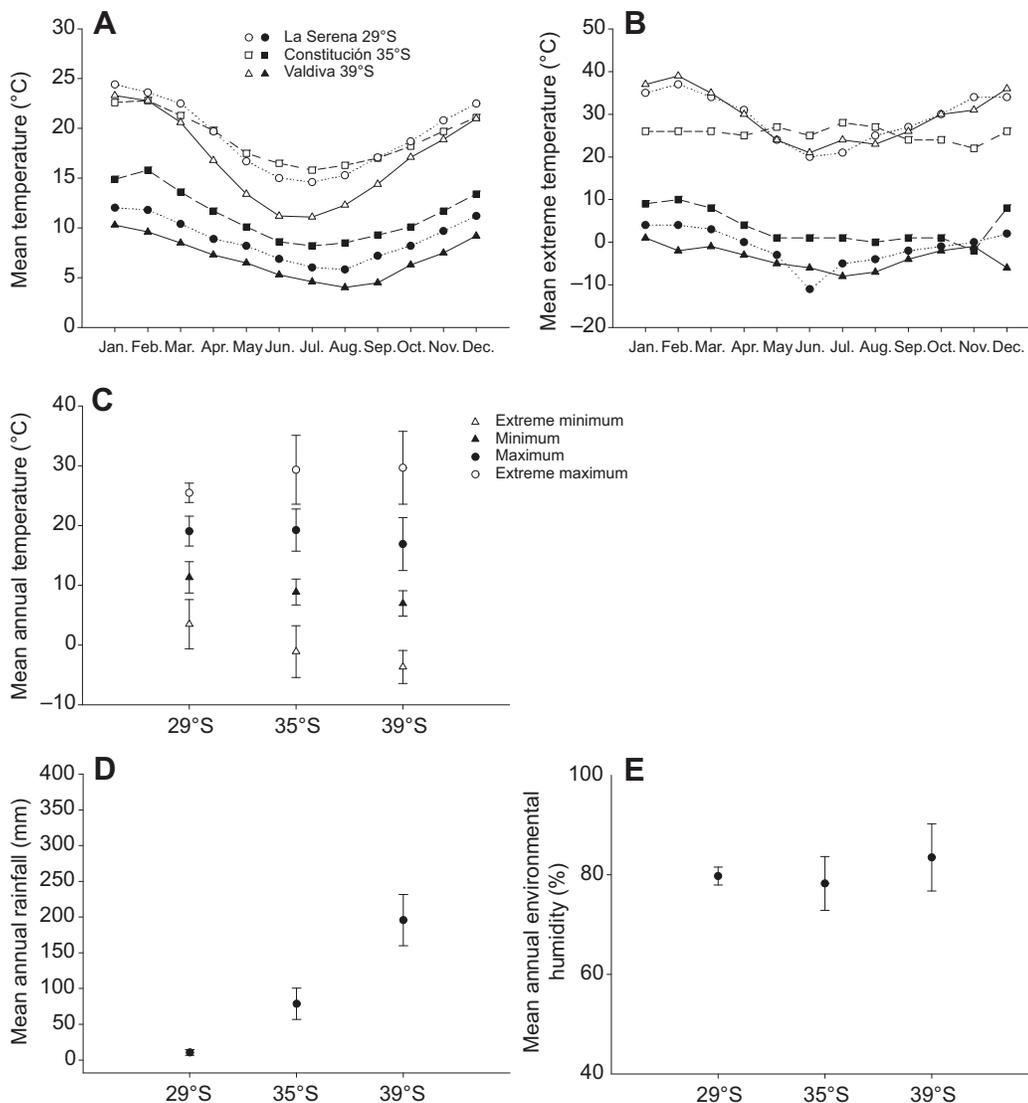
In principle, there are two ways to explain changes in growth rate in ectothermic organisms from different environments. Firstly, as noted above, growth can be maximized by minimizing energy expenditure for maintenance or activity (Pörtner et al., 2005). Alternatively, growth can be maximized when selection promotes increased energy turnover (i.e. maximizing energy extraction), which generates excess energy for growth (Pörtner et al., 2005; Piersma and van Gils, 2011). Unfortunately, neither of these explanations is compatible with our results. In fact, in our study, those animals from the southernmost population, which exhibited higher growth rates, also exhibited lower apparent dry-matter digestibility (i.e. lower digestive efficiency). In a recent study using *C. aspersum* populations in a similar geographical gradient in Chile, Naya et al. found that at 20°C those snails from the highest latitude exhibited lower size and mass of digestive organs compared with lower latitude populations (Naya et al., 2011). This evidence could be associated with the lower dry-matter digestibility that we found in our study, because a smaller digestive tract involves a greater rate of food passage and lower intestinal retention (Karasov and Martínez del Río, 2007). However, in the present study, snails from the highest latitude population grew faster than those from lower latitude populations, which is completely counterintuitive as it is not clear how they could obtain the extra energy (Lindgren and Laurila, 2009).

A Darwinian demon is a hypothetical organism that is able to maximize all elements of its fitness simultaneously. It reproduces immediately after birth, produces the maximum number of young and lives indefinitely (Williams, 1966; Gadgil and Bossert, 1970). As no such organism could exist, this metaphor is frequently used as a null hypothesis to be rejected when trade-offs are detected, or to highlight the fact that our reasoning is incomplete (e.g. because there is some missing variable). In this study, we cannot reject the Darwinian demon hypothesis and we do not believe snails are such 'magic' organisms that maximize everything with no costs. Here, adjustments in energy allocation to the digestive machinery and to protein storage (Piersma and van Gils, 2011), as well as differential nutrient digestibility among populations (Bozinovic et al., 1997), are possible explanations for the puzzling result. Nevertheless, further research is warranted to search for this missing link.

MATERIALS AND METHODS

Populations and climatic data

Three coastal populations of the land snail *C. aspersum* were selected from a latitudinal gradient of ca. 1300 km from northern to southern Chile. We selected these localities based on their different climatic characteristics (Fig. 2) and genetic differentiation (Gaitán-Espitia et al., 2013c). La Serena (29°54'S, 71°15'W) is a mesomediterranean arid environment with a mean annual



temperature of $13.43 \pm 2.17^\circ\text{C}$, whereas Constitución ($35^\circ 20'S$, $72^\circ 25'W$) is a mesomediterranean subhumid environment with a mean annual temperature of $12.36 \pm 2.65^\circ\text{C}$, and Valdivia ($39^\circ 38'S$, $73^\circ 5'W$) is a mesotemperate perhumid environment with a mean annual temperature of $10.79 \pm 3.05^\circ\text{C}$ (Amigo and Ramírez, 1998; Luebert and Plissock, 2006; Naya et al., 2011). Climatic data were downloaded from <http://www.meteochile.gob.cl>.

Common-garden experiment

To ensure that measured traits come from population differences and not phenotypic plasticity or maternal effects, we measured individuals from the third generation after capture, 2 years after field collection. Six-hundred adult snails of approximately the same M_b (mean \pm s.d. 4.21 ± 0.63 g, approximate age less than 1 year) (Gaitán-Espitia et al., 2013b), were collected by hand from gardens and parks in each of the three localities (total of 1800 snails), placed in plastic containers and transferred to the laboratory at the Universidad Austral de Chile in Valdivia. Snails were maintained at densities of 50 animals in plastic cages ($60 \times 60 \times 13$ cm), filled with 10 cm of humid soil, and fed *ad libitum* with a mix of corn–wheat flour and calcium carbonate (1:1:0.3). The temperature and photoperiod for rearing conditions were 20°C and 16 h:8 h light:dark, respectively. This temperature is within the thermal optimum range of these populations (Gaitán-Espitia et al., 2013a). Relative humidity was maintained at high levels by sprinkling the interior of the boxes with water every day. Visual inspections were made during the next 6 months to identify copulation and egg laying. Snails were isolated into laying boxes as soon as they were seen copulating. Eggs were kept at the same environmental conditions, recording the incubation time, size and mass at eclosion. After eclosion, snails were maintained in plastic boxes ($15 \times 8 \times 3$ cm) of 20 individuals during the growth period. Animals were reared until pre-adulthood (i.e. 6 months) in the third generation.

Population growth rate

The growth of snails was estimated from mass-at-age by a Gompertz equation, which has previously been found useful for description of growth rate in the early life stages of molluscs (Hernandez-Llamas and Ratkowsky, 2004). M_b was measured monthly from eclosion until 6 months of age in 1454 animals. Growth rate (ΔG) was analysed following previous methods (Kaufmann, 1981). The rate of growth was estimated for each individual as:

$$[\ln(\text{final mass}) - \ln(\text{initial mass})] / t, \quad (1)$$

where t is time. This growth rate was then regressed against $\ln(m)$, where m is the geometric mean of the final mass and initial mass. In general terms, the elevation of the regression line represents the age-specific growth rate whereas the slope is the rate at which snails approach their asymptotic mass. The slope (a) and intercept on the y -axis (b) of this regression are related as follows:

$$\Delta G = -a \times \ln(m) + b. \quad (2)$$

This linear relationship allows ΔG to be used as a response variable for statistical analyses (Kaufmann, 1981; Moorhouse et al., 2008). The integrated form of this relationship is described by:

$$L_t = L_\infty \times \exp[-W \times \exp(-gt)], \quad (3)$$

where L_t is the M_b at time t ; L_∞ is a constant that represents the asymptotic mass of the snails, $L_\infty = \exp(b/a)$; W is the instantaneous rate of growth at age t_0 ; $W = \ln(L_\infty/L_t)$; g is the growth rate constant (month^{-1}); and t is the time in months (Kaufmann, 1981). The integrated equation can be used to predict the age of an individual from its mass on first capture (Kaufmann, 1981; Moorhouse et al., 2008). These parameters were estimated using TableCurve2D curve-fitting software (version 5.01; Systat Software Inc.). Additionally, we estimated the overall growth rate for each snail, taking the difference in the somatic tissue and body size from eclosion until the sixth month of age.

Energy expenditure and water balance

A total of 1454 pre-adult snails (i.e. 6 months after eclosion) were randomly selected for metabolic analysis. In this study, SMR, the obligatory energetic cost of maintenance in ectotherms, was measured as the rate of carbon dioxide production in a computerized open-flow respirometry system, as

described previously (Gaitán-Espitia et al., 2012). In brief, CO_2 production was measured continuously with an infrared CO_2 analyser (LI-COR 7000, Sable Systems), which was calibrated periodically against two kinds of gas (CO_2 -free air, and a commercial mix of 101 ppm CO_2). The analyser was connected to a computerized data-acquisition system (Expe Data software, Sable Systems), similar to that used elsewhere (Lighton and Turner, 2004). A Sable Systems eight-channel multiplexer was used to make the measurements, five chambers with individual snails and three chambers for baselining (before and after each record), which allowed correction for possible drift (although it was almost non-existent between baselines). All measurements were performed at 20°C , which corresponds to the thermal optimum range of these populations (Gaitán-Espitia et al., 2013a). The arrangement of the respirometry system was as follows: ambient air was first pumped at 100 ml min^{-1} through a Drierite/soda lime column, to remove water vapour and CO_2 . The air was then passed through a single flow meter maintaining a constant ($\pm 1\%$) flow rate through the respirometry chambers. CO_2 -free air was always flowing through all chambers while one of them was being measured. We used transparent metabolic chambers (60 ml), each one with a 100% hydrated snail. Animal activity was visually monitored at intervals of ca. 10 min during measurements, which had a total duration of 45 min each. Activity was rarely observed during the respirometry measurements and the data of active animals were not included in the analysis. Parts per million were transformed to $\text{ml CO}_2 \text{ h}^{-1}$, taking into account the flow rate, by a macroprogram recorded in the ExpeData software (Sable Systems). The respirometry equation used was:

$$\dot{V}_{\text{CO}_2} = \text{STP} \times (F_{\text{ECO}_2} - F_{\text{ICO}_2}) \times \dot{V} / \{1 - F_{\text{ECO}_2} \times [1 - (1 / \text{RQ})]\}, \quad (4)$$

where F_{ECO_2} is the excurrent fractional concentration of CO_2 , F_{ICO_2} is the input fractional concentration of CO_2 , \dot{V} is the flow rate in ml min^{-1} , STP is the correction factor for standard conditions of temperature and pressure (which for mass flowmeters is equal to 1) and RQ is the respiratory interchange ratio (the respiratory quotient), which was assumed equal to 0.85.

To avoid any noise or erroneous recordings generated by animal manipulation, we eliminated the first 10 min of the record (600 samples) (Gaitán-Espitia et al., 2012). From each individual record, we extracted the average of each transformed record ($\dot{V}_{\text{CO}_2, \text{mean}}$) which is used here as a proxy of SMR. To achieve a post-absorptive state, metabolic rate was measured in individuals deprived of food for 18 h (Naya et al., 2011). All metabolic trials were performed during the day, when land snails are inactive, which corresponds to the rest phase in this species (J.D.G.-E. and R.N., personal observation). All individuals were weighed at the beginning and at the end of the test period (i.e. 45 min), recording the mean M_b . Finally, to determine the extent of evaporative water loss (i.e. body dehydration), the snails were weighed at the beginning of each trial, when the animals were fully hydrated, and at the end of the test period (i.e. 45 min), recording the mean M_b and also the differences between initial and final M_b as a proxy for body water loss (Gaitán-Espitia et al., 2012).

Food consumption and digestible energy intake

After the 1454 respirometric trials, snails were maintained for 5 days in laboratory conditions with a diet of 100% lettuce to eliminate any remnants of the previous diet (i.e. flour mix) in the intestine. Animals were then deprived of food for 2 days and starting on the third day they were provided with a known amount of wheat flour for 15 days. On day 16 they were exposed to the 100% lettuce diet for three more days. Every day, faeces were removed, dried and weighed at 72°C to a constant mass (± 0.0001 g). At the end of the experiment apparent dry-matter digestibility was calculated as $(I-E)/I$, where I is food intake and E is egestion (both in g). Digestibility is apparent because faecal contributions of endogenous protein and gut microflora were not considered (Lardies et al., 2004b). Energy content of food and faeces were determined in a Parr 1261 computerized calorimeter (Parr Instrument Company, Moline, IL, USA). Because there was no difference in the faecal energy content among populations, we excluded this variable from the analysis.

Statistical analysis

To evaluate the correlation between M_b , growth rate, energy expenditure (i.e. SMR), the ability to maintain water balance (i.e. body dehydration) and the

apparent dry-matter digestibility in *C. aspersum* among populations in the latitudinal gradient, we conducted Pearson's correlation test. Maximum likelihood estimates of Gompertz growth rate parameters for each population were obtained using TableCurve2D curve-fitting software (version 5.01; Systat Software Inc.) Growth rate curves were compared based on the confidence intervals of the parameters and their slopes were tested using the maximum likelihood test (Kimura, 1980). Comparisons of size and mass of eggs and hatchlings, as well as M_b , body size, growth rate, body dehydration and dry-matter digestibility in pre-adults among populations were done using ANOVA, whereas SMR was analysed by ANCOVA with mean M_b as a covariate. When differences in the means were significant at the $P < 0.05$ level, an *a posteriori* Tukey (HSD) test was used to locate significant differences among pairs. We checked normality and homoscedasticity by the Lilliefors and Levene tests, respectively. All data were square-root or \log_{10} transformed before the analysis. These analyses were run in Statistica v7.0 software (StatSoft).

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Competing interests

The authors declare no competing financial interests.

Author contributions

J.D.G.E. and R.N. conceived and designed the experiments. J.D.G.E. performed the experiments. J.D.G.E. analysed the data. J.D.G.E. and R.N. wrote the manuscript.

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