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Exploring the functional association between physiological plasticity, climatic variability, and geographical latitude: lessons from land snails

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ABSTRACT

Background: The climatic variability hypothesis states that, as the range of climatic fluctuation experienced by terrestrial animals increases with latitude, individuals at higher latitudes should be more plastic than individuals inhabiting lower latitudes. However, it is unclear whether comparatively high flexibility at higher latitudes is due to the direct effect of climatic variability or to other factors associated with latitude.

Aim: To investigate the relationship between phenotypic flexibility, geographical latitude, and climatic variability using a dataset where latitude and climatic variability are inversely related.

Methods: We assessed the physiological plasticity to cope with thermal change (10°C vs. 20°C), at the level of metabolic rate and organ dry weight, in three populations of the brown garden snail (*Cornu aspersum*): Viña del Mar (33°20′S, 71°32′W), with high temperature and rainfall variability; Concepción (36°47′S, 73°7′W), with a narrow range of temperature variability and intermediate rainfall variability; and Valdivia (39°38′S, 73°5′W), with low temperature and rainfall variability.

Results: Standard metabolic rate was higher at 20°C than at 10°C, but did not differ between populations. Intestine dry weight did not differ among populations but it was higher at 20°C than at 10°C, particularly for individuals from the Viña del Mar and Concepción populations. Hepatopancreas and kidney dry weight differed between populations, which was due to higher values in Viña del Mar at 20°C.

Conclusions: Flexibility in the weight of the organs analysed changed in a similar fashion to annual temperature variation at each locality, suggesting that, as stated by the climatic variability hypothesis, climatic variability is the main force behind physiological plasticity.

Keywords: climatic variability hypothesis, *Cornu aspersum*, digestive flexibility, macrophysiology, metabolic rate, phenotypic plasticity.

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INTRODUCTION

In 1967, Daniel Janzen proposed the climatic variability hypothesis (CVH), which states that, as the range of climatic fluctuation experienced by terrestrial animals increases with latitude (or altitude), individuals at higher latitudes require broader tolerance ranges than individuals inhabiting lower latitudes (Janzen, 1967). A number of authors have suggested that an adaptive response of animals to such climatic variability is to increase the amplitude of functional capacities (i.e. physiological plasticity), especially physiological variables related to performance, such as nutrient acquisition and processing rates (see Piersma and van Gils, 2010). Hence, according to the CVH, physiological plasticity should increase with latitude (Stevens, 1989; Chown *et al.*, 2004). Over the last four decades, the CVH has been evaluated by comparing the physiological plasticity of different populations and species along latitudinal gradients (Ghalambor *et al.*, 2006; Gaston *et al.*, 2009; Bozinovic *et al.*, 2011). These studies, mostly restricted to the analysis of thermal tolerance and acclimation abilities in ectothermic species, have demonstrated an increase in phenotypic flexibility with latitude [e.g. insects (Levins, 1969; Kimura, 1988; Hoffman and Watson, 1993; Addo-Bediako *et al.*, 2000); amphibians (Brattstrom, 1968; Snyder and Weathers, 1975; Feder, 1982); lizards (Tsuji, 1988; van Berkum, 1988; Cruz *et al.*, 2005)].

In most of the aforementioned studies, however, latitude was used as the predictor variable, instead of directly using climatic data. Moreover, those studies that tested the fit of both latitude and climatic variability data found that latitude was a better predictor of phenotypic variation than climate. Some explanations currently given for this result include: (1) latitude could be a better predictor of long-term regimes of climatic variables than contemporary records of weather stations; (2) latitude is correlated with several other ecologically relevant factors, such as day length and environmental productivity, in addition to climatic variables; (3) latitude is also related to several historical factors, such as the number of available habitats and biogeographical boundaries (Gaston *et al.*, 1998; Rezende *et al.*, 2004; Naya *et al.*, 2008). In fact, the general association between latitude and climate does not permit separation of these factors to establish to what extent higher flexibility at higher latitudes is due to the direct effect of climatic variability or to other factors associated with latitude (Ghalambor *et al.*, 2006). In this scenario, an experiment to examine the relationship between physiological plasticity, latitude, and climatic variability for a region where latitude and climatic variability are inversely correlated or, at least, uncoupled, would be of value.

One of the most universal forms of physiological plasticity is the ability to modify nutrient processing and absorption capacity in a changing environment, a phenomenon that has been widely reported for vertebrate species (Pennisi, 2005; Karasov *et al.*, 2011). Invertebrates, however, have received limited attention in this respect. Whereas phenotypic plasticity in functional morphology and its metabolic consequences have been widely studied in many groups of invertebrates (e.g. Brakefield *et al.*, 1998; Fox *et al.*, 1999; Gotthard *et al.*, 1999; Sack and Stern, 2007), we could find few studies addressing physiological plasticity of intestinal capacity (Yang and Joern, 1994; Bock and Mayer, 1999; Labarta *et al.*, 2002; Fernandez-Reiriz *et al.*, 2005; Gao *et al.*, 2008).

Accordingly, the aim of the present study was to advance our understanding of the causal link between physiological plasticity and latitude in terrestrial invertebrates, taking advantage of a species distributed over a latitudinal gradient where latitude is inversely correlated with climatic fluctuations. We studied the pattern of physiological plasticity (at the level of metabolic rate and weight of nutrient processing organs) in three populations of the land snail *Cornu aspersum* (syn. *Helix aspersa*) in southern

South America. We predicted that if climatic variability is the major cause of physiological plasticity, an inverse relationship between flexibility and latitude would be observed for our data set.

METHODS

Populations and climatic data

Although *C. aspersum* is an introduced species in Chile, the time elapsed since its introduction (some time during the European colonization of the Americas) can be considered long enough to allow local adaptation to have occurred (see Bradshaw and Holzapfel, 2006). We studied three coastal populations of *C. aspersum* in Chile within a latitudinal range of ~900 km: Viña del Mar (33°20′S, 71°32′W), Concepción (36°47′S, 73°7′W), and Valdivia (39°38′S, 73°5′W) (Fig. 1A). We selected these localities based on their climatic

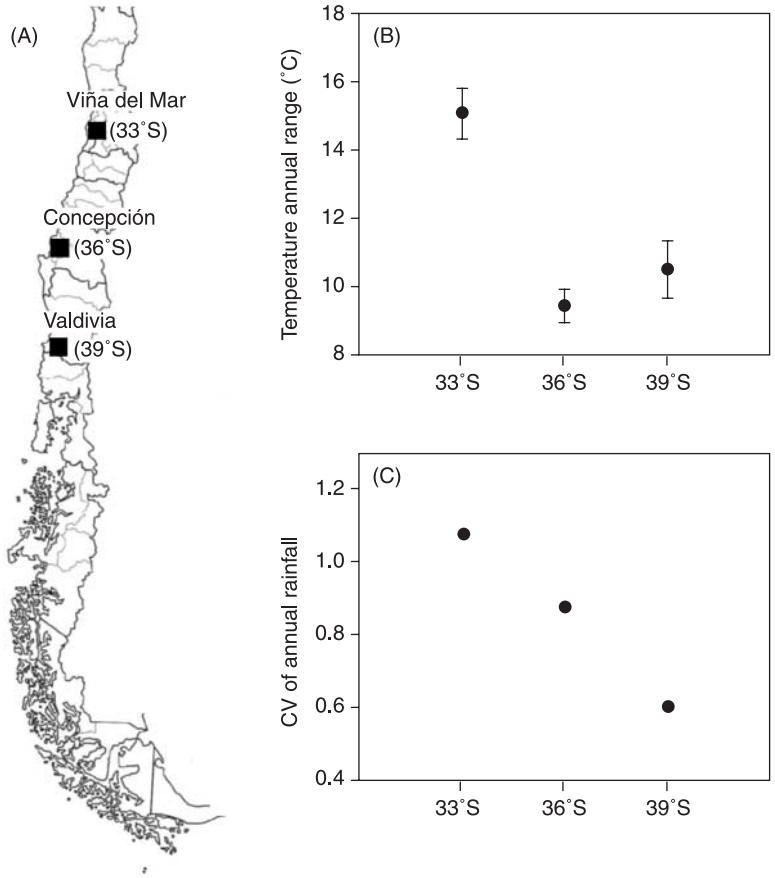

Fig. 1. Map of Chile showing the locations of the three populations studied (A), and the annual range of temperature (B) and the rainfall coefficient of variation (C) for Viña del Mar (33°S), Concepción (36°S), and Valdivia (39°S).

characteristics: Viña del Mar has a mean temperature of 14.8 ± 1.3°C and mean annual rainfall of 190 mm; Concepción has a mean temperature of 13.0 ± 0.8°C and mean annual rainfall of 790 mm; and Valdivia has a mean temperature of 11.7 ± 1.0°C and mean annual rainfall of 1120 mm. In terms of climatic variability, the annual range of temperature is greater for Viña del Mar than for Concepción and Valdivia, while the rainfall coefficient of variation decreases from Viña del Mar to Concepción to Valdivia (Figs. 1B and 1C). Thus, temperature and rainfall variability covaried in our data set, avoiding further exploration of the effect of each climatic variable on physiological plasticity. Climatic data were downloaded from http://clima.msn.com/, which is based on the Foreca database (Foreca Ltd., Helsinki, Finland).

Animal collection and experimental design

Adult individuals of *C. aspersum* were collected by hand from gardens and parks in Viña del Mar ($n = 20$), Concepción ($n = 20$), and Valdivia ($n = 20$), placed in plastic containers, and transported to the laboratory in Valdivia, one day after collection. Individuals were randomly assigned to one of two environmental temperatures: 10 ± 1°C or 20 ± 1°C (mean ± range). Subsequently, animals were placed in plastic containers (30 × 15 × 13 cm) with 5 cm of humid litter soil, and reared on food (rabbit chow and maize starch mixed with lime for shell maintenance) and water *ad libitum* for 3 months. During the experiment, photoperiod was fixed to 14 h light/10 h dark.

Determination of metabolic rate

In this study, standard metabolic rate (SMR), the obligatory energetic cost of maintenance in ectotherms, was measured as the rate of carbon dioxide production within an open system, as described by Nespolo *et al.* (2007). In brief, CO_2 production was measured continuously with an infra-red CO_2 analyser (LI-COR LI6262, Lincoln, NB, USA) capable of resolving differences of one part per million of CO_2 in air. The analyser was calibrated periodically against two types of gas (CO_2-free air and a commercial mix of 291 ppm of CO_2). Although there was almost no drift between calibrations, we performed baseline measurements before and after each recording. The arrangement of the respirometry system was as follows: air was pumped at a rate of 120 $ml \cdot min^{-1}$ through a Drierite–soda lime–Drierite column, then through a flow meter that maintained flow rate within ±1% of the desired rate, and a transparent respirometry chamber with a volume of 60 ml. Due to the use of free CO_2 air, baseline measurements have no drift and noise is considerably reduced. We used a multiplexer to locate each animal in a different, isolated metabolic chamber, of which there were eight (three of which were empty, used as blanks). Carbon dioxide production was measured over a 45-min period, but SMR was estimated as the total average of the last 30 min of each recording. All metabolic trials were performed at the acclimation temperature (10°C or 20°C) and during the day, which in this species corresponds to the resting daily period (Bailey, 1975). In addition, animals were deprived of food for 18 h before measurements were made, which was enough time to attain a post-absorptive state based on preliminary measurements of mean retention time in this species (Artacho and Nespolo, 2009). Also, animal activity was visually monitored at intervals of ~10 min. The few recordings of animals that were active during the metabolic measurements were discarded.

Given that the analyser provides parts-per-thousands, and SMR should be reported as a rate, we transformed the recordings as follows. From the respirometric recordings and based on the configuration of the system (i.e. the flowmeter was upstream from the chamber and both CO_2 and water were 'scrubbed'), we computed the following variables (see Withers, 1977):

$$V\mathrm{CO_2}\ (\mathrm{ml\ CO_2 \cdot min^{-1}}) = (\mathrm{F_eCO_2} \times \mathrm{FR}) / [(1 - \mathrm{F_eCO_2}) \times (1 - 1/\mathrm{RQ})]$$

where $V\mathrm{CO_2}$ is the rate of CO_2 production, $\mathrm{F_eCO_2}$ is the excurrent fractional concentration of CO_2, FR is flow rate ($\mathrm{ml \cdot min^{-1}}$), and RQ is the respiratory quotient, assumed to be equal to 0.85 in herbivorous animals (Rogowitz and Chappell, 2000). Carbon dioxide production was converted into energetic units using the energy equivalent of 20.92 $\mathrm{J \cdot ml^{-1}}$ CO_2 (Walsberg and Wolf, 1995).

Organ size measurements

The day after metabolic determinations, snails were sacrificed by immersion for 12 h and then they were dissected. We isolated the digestive tube, hepatopancreas, and kidneys. After dissection, the digestive tube was washed and perfused. All the organs were dried at 60°C to constant mass (72 h), together with the animals' shell and remaining organs (i.e. carcass). We obtained dry weight of organs using an electronic balance (Chyo JK180; ± 0.0001 g).

Statistical analysis

Differences in body size (m_b) between populations and temperatures were evaluated by two-way analyses of variance (ANOVA). Differences in SMR, intestine, hepatopancreas, and kidney dry weight were evaluated separately by two-way analyses of covariance (ANCOVA), using body size (for SMR) or carcass dry weight (for weight of organs) as covariates. Before each statistical analysis, data were examined for assumptions of normality and homogeneity of variance, using the Kolmogorov-Smirnov and Levene test, respectively. In some cases, data were log-transformed (e.g. SMR, intestine and kidney dry weight), square root transformed (e.g. body mass) or transformed to the inverse (e.g. hepatopancreas dry weight) to meet the assumptions of the analyses. Interactions between covariates and factors were tested using a parallelism test. The results are presented as least square adjusted means ± 1 standard error, and statistical significance was set at $P < 0.05$. All analyses were performed using the statistical package Statistica® (2004) v.6.1 for the Windows operating system.

RESULTS

Body mass and shell mass did not differ between populations or between environmental temperatures (Tables 1 and 2). Standard metabolic rate did not differ between populations, but, as expected, it was higher at 20°C than at 10°C (Tables 1 and 2). Assuming a respiratory quotient of 0.85, the mass-specific SMR for the three populations at 10°C was 31.53 μW, whereas at 20°C it was 825.7 μW. Exploration of the functional correlation between organ sizes and metabolic rates showed that only intestine dry weight was significantly correlated with SMR ($r = 0.46$, $P < 0.05$).

In terms of organ size, intestine dry weight did not differ among populations, but it was significantly higher at 20°C than at 10°C (Tables 1 and 2), in particular for individuals from

Table 1. Variables measured at each of two temperatures for the three populations studied

	Viña del Mar			Concepción			Valdivia		
	mean	S.E.	*n*	mean	S.E.	*n*	mean	S.E.	*n*
10°C									
Body mass (g)	7.14	0.42	10	6.57	0.35	10	6.92	0.33	10
Shell mass (g)	1.44	0.14	6	1.22	0.08	9	1.28	0.08	10
SMR ($J \cdot h^{-1}$)	6.03	0.88	10	5.25	0.89	10	5.60	0.88	10
Intestine dry mass (g)	0.0100	0.0054	6	0.0102	0.0044	9	0.0126	0.0042	10
Hepatopancreas dry mass (g)	0.0511	0.0088	6	0.0642	0.0072	9	0.0437	0.0068	10
Kidney dry mass (g)	0.0484	0.0120	6	0.0302	0.0098	9	0.0325	0.0092	10
20°C									
Body mass (g)	7.90	0.56	10	7.34	0.75	10	6.70	0.40	10
Shell mass (g)	1.25	0.16	9	1.13	0.08	10	1.18	0.08	10
SMR ($J \cdot h^{-1}$)	10.63	0.90	10	9.98	0.08	10	11.12	0.88	10
Intestine dry mass (g)	0.0319	0.0053	9	0.0302	0.0044	10	0.0231	0.0045	9
Hepatopancreas dry mass (g)	0.0783	0.0087	9	0.0481	0.0071	10	0.0394	0.0070	10
Kidney dry mass (g)	0.0812	0.0117	9	0.0331	0.0097	10	0.0392	0.0099	9

Note: Values are least square absolute means ± 1 standard error (for body mass and shell mass) or least square adjusted means ± 1 standard error (for standard metabolic rate and organ weights).

Table 2. Results of the two-way analyses of variance (for body mass and shell mass) and covariance (for standard metabolic rate and organ dry masses)

	MS	d.f.	*F*	*P*
Body mass				
Population	0.09	2	1.16	0.32
Temperature	0.07	1	0.92	0.34
Population × Temperature	0.05	2	0.63	0.54
Error	0.08	54		
Shell mass				
Population	0.02	2	0.93	0.40
Temperature	0.05	1	2.46	0.12
Population × Temperature	0.003	2	0.14	0.87
Error	0.02	48		
SMR				
Population	0.01	2	0.58	0.56
Temperature	1.18	1	58.56	<0.0001
Population × Temperature	0.01	2	0.48	0.62
Error	0.02	53		
Intestine dry mass				
Population	0.17	2	0.60	0.55
Temperature	9.66	1	34.6	<0.0001
Population × Temperature	0.82	2	2.94	0.06
Error	0.28	46		
Hepatopancreas dry mass				
Population	190.3	2	4.49	0.02
Temperature	31.4	1	0.74	0.39
Population × Temperature	24.9	2	0.59	0.56
Error	42.4	47		
Kidney dry mass				
Population	1.95	2	5.44	<0.01
Temperature	0.70	1	1.96	0.17
Population × Temperature	0.13	2	0.37	0.69
Error	0.36	46		

Note: MS = mean square; d.f. = degrees of freedom.

the Viña del Mar and Concepción populations (Fig. 2A). In fact, there was an approximate 200% change in intestine dry weight with temperature in these two populations. In contrast, hepatopancreas and kidney dry weight were not affected by temperature, but did differ between populations (Tables 1 and 2); these differences between populations were mainly due to the higher values observed in Viña del Mar at 20°C (Fig. 2B, C).

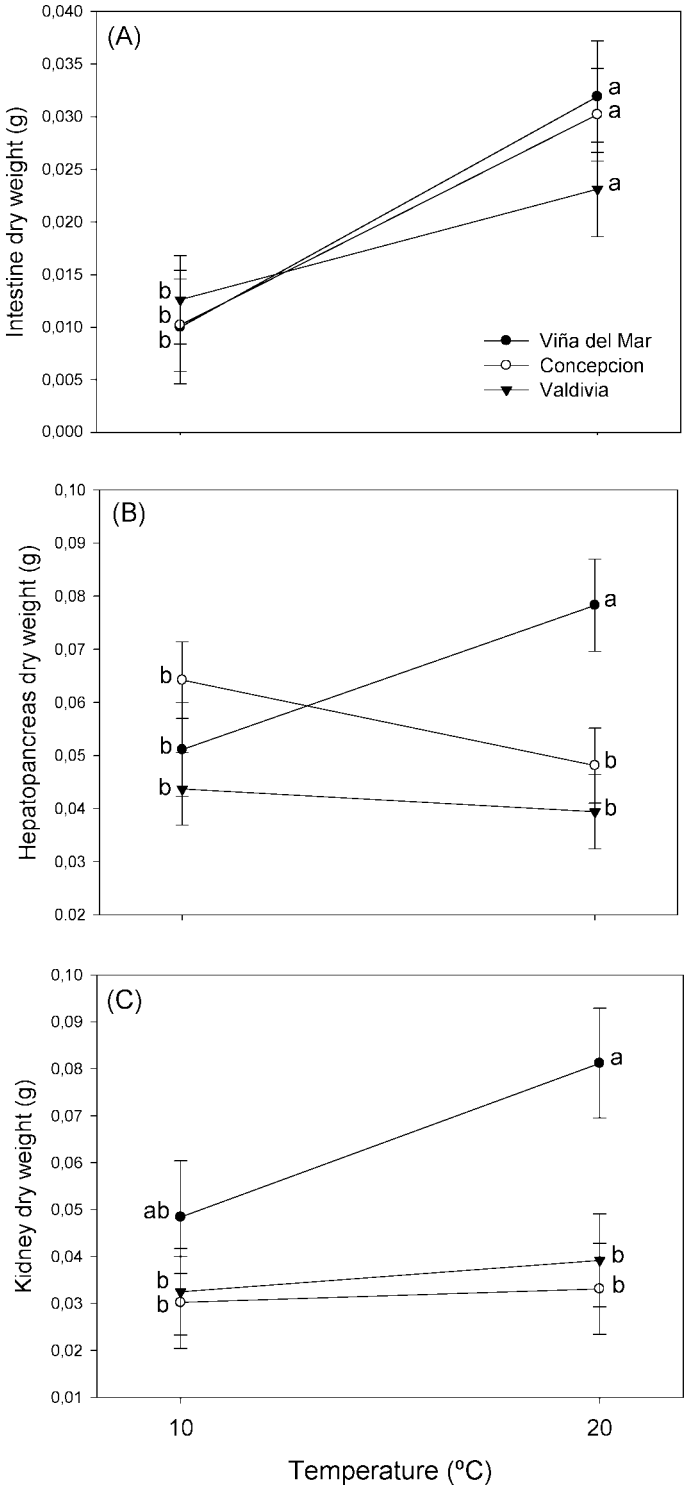

Fig. 2. Thermal reaction norms for intestine (A), hepatopancreas (B), and kidney (C) dry weight for each population. Values presented are least square adjusted means ± 1 standard error (bar). Different letters indicate differences among groups (after a Tukey HSD test).

DISCUSSION

Although recent research has suggested that some populations are able to undergo rapid responses via natural selection (see Bradshaw and Holzapfel, 2006), it is accepted that for most species to cope, the accelerated change in environmental conditions (i.e. global change) will be closely related to the amount of plasticity for fitness-related traits (Berteaux *et al.*, 2004; Charmentier *et al.*, 2008; Deutsch *et al.*, 2008; Gienapp *et al.*, 2008; Teplitsky *et al.*, 2008; Fuller *et al.*, 2010). In this context, determining the level of phenotypic flexibility that currently exists in natural populations, and identifying global patterns of phenotypic flexibility are of paramount importance. However, few studies have addressed the amount of phenotypic flexibility expressed by populations of the same species occupying different habitats (but see Nunez-Olivera *et al.*, 1996; James *et al.*, 1997; Santamaria *et al.*, 2003; Gaston *et al.*, 2009; Bozinovic *et al.*, 2010; Crispo and Chapman, 2010).

Although our dataset did not permit formal statistical evaluation of the correlation between physiological plasticity and climatic variability, we found that snails from populations that inhabit environments with broader ranges of climate variability tend to be more flexible in their anatomical features than snails from populations that inhabit more stable environments. In this sense, two interesting results emerge from the comparisons of organ dry weight among populations. First, flexibility in the weight of the three organs analysed here paralleled the annual range of temperature, being greater in the snails from Viña del Mar compared with snails from the other two populations. This strongly suggests that climatic variability, and not geographic latitude, is the main force behind physiological plasticity. Second, the greater flexibility observed for the Viña del Mar population was mainly due to greater organ size at 20°C. Given that monthly mean temperature reaches 20°C from December to February in Viña del Mar, but never rises above 17°C in the other two localities, our results support the idea that physiological plasticity allows animals to cope with climatic variability.

In contrast to organ size, there were no differences in SMR that were associated with the latitudinal cline and/or variation in annual temperature range among populations. This result is consistent with other studies in land snails (Artacho and Nespolo, 2009) and also in other invertebrates where energy metabolism does not change among populations (Ashby, 1997; Nespolo *et al.*, 2003). In addition, the lack of differences in SMR between populations at each of the two temperatures was consistent with the thermal sensitivity (Q_{10}), which was very similar for the three populations, ranging from 1.76 (Viña del Mar) to 1.98 (Valdivia). Thus, the fact that Q_{10} was a little higher for Valdivia than for the other two populations is consistent with thermodynamic considerations for general biochemical reactions, where Q_{10} is predicted to be higher in populations living at lower temperatures (Schmidt-Nielsen, 1995). On the other hand, SMR was markedly affected by environmental temperature, which is not surprising given the profound effects of temperature in nearly all physiological and biochemical processes in ectothermic species (Cossins and Bowler, 1987; Huey and Berrigan, 2001).

Finally, it is noteworthy that in contrast to vertebrates – for which the digestive system is considered a model system for the study of physiological plasticity (for recent reviews, see McWilliams and Karasov, 2001; Naya and Bozinovic, 2004; Starck, 2005; Naya *et al.*, 2007; Karasov *et al.*, 2011) – digestive flexibility has been little investigated in invertebrates (but see Yang and Joern, 1994; Bock and Mayer, 1999; Labarta *et al.*, 2002; Fernandez-Reiriz *et al.*, 2005; Gao *et al.*, 2008). Thus, we believe that the present study represents an important contribution by showing that land snails exhibit an impressive physiological plasticity of the digestive tract, as some individuals increase the size of this organ three-fold after thermal acclimation. Given the relationship between maintenance

costs and temperature in ectothermic animals, an increase in food consumption concomitant with a rise in environmental temperature should be expected. Thus, we suspect that the observed increase in intestinal capacity at 20°C may allow animals to cope with higher food intake, without a noticeable decrease in their digestive efficiency. However, exploration of the functional correlation among organ sizes and metabolic rates showed that the enlargement of the intestine was significantly correlated with SMR. Therefore, our results also indicate that the better performance conferred by a larger digestive machinery is costly to maintain.

In summary, our results suggest that climatic variability, and not geographic latitude, is the main force behind physiological plasticity. Only three populations were included in our design as it would be difficult to obtain a larger dataset for which climatic variability and latitude are inversely related. Interestingly, a recent study that analysed digestive flexibility in three populations of a bird species (*Zonotrichia capensis*) for which climatic variability was not related to latitude, also found that digestive flexibility was correlated with environmental variability but not with latitude (Maldonado *et al.*, 2011). Although the results of the two studies are congruent, further research is required into the relationship between physiological plasticity, climate, and latitude.

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