

Energy expenditure and body size are targets of natural selection across a wide geographic range, in a terrestrial invertebrate

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Summary

1. One of the central questions in evolutionary ecology is how different functional capacities impact fitness, and how it varies across populations. For instance, do phenotypic attributes influence fitness similarly across geographic gradients? Which traits (physiological, morphological and life history) are most likely to be targets of natural selection? Do particular combinations of traits maximize fitness?

2. In a semi-natural experiment, we analysed introduced populations of an invasive species, the garden snail (*Cornu aspersum*) in Chile, which show low levels of genetic differentiation in spite of the distance. Specifically, we addressed whether the magnitude, sign and form of selection in snail populations could explain the differentiation (or its absence) among populations.

3. A common garden/reciprocal transplant experiment was performed in three populations (La Serena, Constitución and Valdivia) that span a 1300-km latitudinal gradient and differ markedly in climate (semi-arid north to humid south). We released *c.* 450 individuals per population (two generations after field-captured snails) in replicated enclosures at the range extremes (La Serena and Valdivia). Morphological (size and shell darkness), physiological (standard metabolic rate and digestive efficiency) and life-history [growth rate (GR)] traits were measured in all snails before the release. Survival was recorded monthly during 1 year.

4. Survival was significantly higher in snails from La Serena than in snails from Constitución and Valdivia, when raised at La Serena. However, at Valdivia, survival was not different among source populations. Interestingly, we found negative correlational selection in M_B and SMR_R at La Serena, whereas at Valdivia we only found directional selection on GR and M_B , and stabilizing selection on SMR_R .

5. These results suggest that selection on physiological traits related with energy allocation is pervasive, irrespective of climate and distance.

Key-words: adaptation, bioenergetics, climate, geographic range, invasive species, natural selection

Introduction

Knowing the form, sign and magnitude of selection on functional traits (i.e. those attributes that influence the rate of energy conversion from nutrients to biological work,

which finally impacts fitness) is important since the different functions that describe how organisms extract, process, allocate and spend energy – such as rates of foraging, digestion, metabolic activity, growth and reproduction – could influence how adaptations arise (Nespolo *et al.* 2005; Violle *et al.* 2007; Czarnoleski *et al.* 2008; Naya, Bozinovic & Karasov 2008; Bruning *et al.* 2013). However,

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these are context-dependent processes that depend on a number of factors that vary across the geographic range of species (see below).

Within the geographic range, populations can be isolated or highly interconnected, which determines to a great extent the degree of differentiation or homogenization they exhibit (Brown, Stevens & Kaufman 1996). Whereas the demographic and historical processes explaining population differentiation have received much attention (e.g. Gubitz, Thorpe & Malhotra 2000; Guiller *et al.* 2001; Calsbeek & Irschick 2007; Carstens & Knowles 2007), how differentiation in functional traits occurs is less understood. The magnitude and form of natural selection, as well as the identity of phenotypic attributes that impact fitness in a given environment, are important determinants of adaptive processes (Wade & Kalisz 1990). Thus, it is of importance to know which traits (physiological, morphological, life history; Fig. 1) are targets of selection across populations. One way to address this is by phenotypic selection studies, in which traits and fitness (or a surrogate) is measured in a sample of individuals (Brodie, Moore & Janzen 1995; Brodie & Janzen 1996; Young, Brodie & Brodie 2004). Nevertheless, whereas phenotypic selection studies in morphological, phenological and life-history traits are abundant (see Endler 1986; Kingsolver *et al.* 2012b), cases of selection on performance and physiological traits are still scarce and have been carried out in a small subset of taxa (Irschick *et al.* 2008; Kingsolver *et al.* 2012b) (see below).

Thousands of phenotypic selection estimates on morphological attributes such as body mass, size, colour and shape have been published to date (reviewed in Kingsolver *et al.* 2012b). These results suggest that selection on morphology is usually strong but variable in shape and magnitude. This variation depends on a number of factors, such as environmental conditions and the involved biological function. Fewer studies have been performed in physiological/performance traits (Benkman & Miller 1996; Kingsolver *et al.* 2001, 2012a; Irschick *et al.* 2008), although metabolic traits related to the energetic cost of maintenance (i.e. resting or standard metabolic rate) have received some attention. For instance, it has been found that selection promotes the reduction of metabolism (i.e. negative association with fitness) in juvenile garden snails (Artacho & Nespolo 2009), radiated shanny larvae (Bochdanský *et al.* 2005), brown trouts (Cano & Nieceza 2006), North American red squirrels (Larivee *et al.* 2010), bank voles (Boratynski *et al.* 2013) and ferns (Saldana *et al.* 2007). These results contrast with studies that found positive associations between metabolism and fitness (Hayes & O'Connor 1999; Jackson, Trayhurn & Speakman 2001; Millidine, Armstrong & Metcalfe 2009), and other studies have not found a consistent association (Boratynski *et al.* 2010; Schimpf, Matthews & White 2012). Phenotypic selection on growth rate (GR) has been determined in a number of organisms, including natural populations of mammals (Loehr *et al.* 2007), fish (Carlson, Hendry &

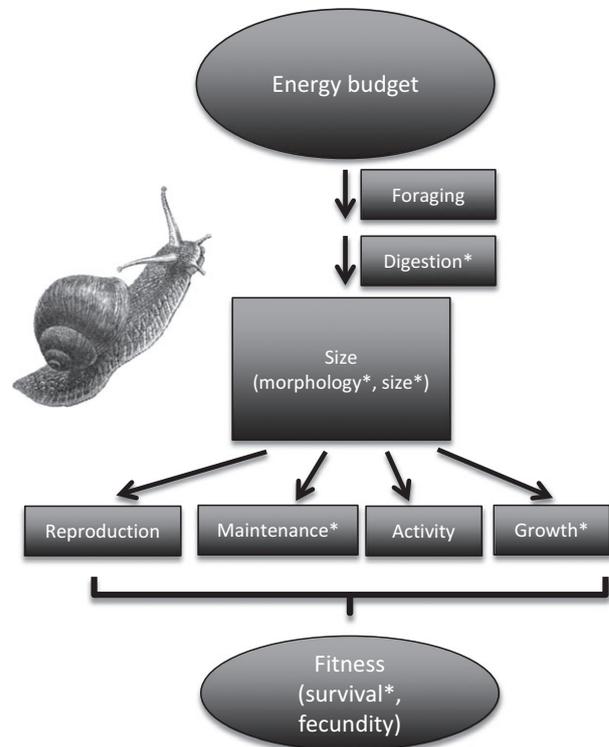


Fig. 1. The underlying model of energy allocation in physiological ecology (Cody 1966; Gadgil & Bossert 1970; Weiner 1992). This model considers that a limited amount of energy is provided by nutrients from the environment (energy budget). Nutrient extraction (foraging, digestion) reduces the usable energy available for vital processes such as reproduction, maintenance, activity and growth. Any of these functions (often measured as rates of energy conversion) can, in principle, be empirically determined in populations to characterize their impact on fitness. Asterisks denote which variables were measured in this study (dry-matter digestibility as a proxy of digestion efficiency; darkness as a proxy of morphology; body mass as a proxy of size; standard metabolic rate as a proxy of maintenance costs; mass growth rate as a proxy of growth; and survival as a proxy of fitness). See text for details.

Letcher 2004), insects (Santos *et al.* 1997) and plants (Salgado-Luarte & Gianoli 2012), showing a range of responses. In contrast, the magnitude and form of selection on traits related to the capacity for nutrient extraction is less known (Illius *et al.* 1995b; Pfaender, Schliwen & Herder 2010). For instance, some indirect measurements have shown that foraging efficiency could have fitness consequences in fish (Millidine, Armstrong & Metcalfe 2009; Krause & Liesenjohann 2012), birds (Benkman 2003) and animals of commercial interest (Nkrumah *et al.* 2006).

Here, we report the results of a semi-natural experiment performed between 2009 and 2012, in populations of a terrestrial mollusc, the invasive snail *Cornu aspersum* (formerly *Helix aspersa*), which distributes across 1300 km in Chile. We applied the 'local vs. foreign criterion' for testing local adaptation using reciprocal transplants (Kawecki & Ebert 2004). This snail has a Mediterranean origin but currently is distributed world-wide; *C. aspersum* invasion occurred in two main waves, about 200 years ago, and it is

continuously introduced and dispersed throughout its introduced range (Guiller *et al.* 2012; Gaitan-Espitia *et al.* 2013). We studied three populations of *C. aspersum* in Chile using individuals that were bred under a common garden design, encompassing three generations. Snails were measured and marked and then released in replicated enclosures at the extremes of its geographic distribution gradient, which markedly differ in climate (see below). They were monitored during a year to record survival (longevity, see Materials and methods for details) as a proxy of fitness. We chose digestive efficiency (=digestibility, the relative rate of nutrient extraction) and GR as functional traits of adaptive significance. We also assessed standard metabolic rate as a typical measure of a physiological cost, which in snails is target of directional/stabilizing selection (Artacho & Nespolo 2009). These snail populations show low differentiation in life-history and morphological traits across its introduced range (Artacho & Nespolo 2008; Naya *et al.* 2011; Bruning *et al.* 2013) and low additive genetic variation (Bruning *et al.* 2013; Nespolo *et al.* 2014). Hence, some population-level processes are probably acting against local adaptation and/or differentiation.

In this study, we addressed whether the magnitude, sign and form of selection in snail populations could explain the observed lack of differentiation among populations. Specifically, and given the range of traits we measured, we tested whether the shape and magnitude of selection differ at those extreme habitats, as well as the target traits. Several scenarios are possible, considering all the combinations of traits and possible selection shapes and magnitudes. Here, we mention one possibility, based on the energy allocation scheme of Fig. 1. At the warmer, drier and also less productive climate of the north, it is expected to find selection promoting higher digestive efficiencies, smaller body sizes and lower metabolism compared with the colder, humid and more productive climate of the south, where selection should promote larger individuals and higher GRs. The finding, on the contrary, of similar traits under selection in both extremes would be suggesting a general rule for energy optimization.

Materials and methods

STUDY ORGANISM

The common garden snail *C. aspersum* (formerly *H. aspersa*) is native to Mediterranean countries (Guiller & Madec 2010) and represents an anthropochorous taxon (a species with human-mediated dispersion) with a widespread distribution in zones with Mediterranean, temperate and subtropical climates (Guiller *et al.* 2001). This land snail exhibits great variation in shell colour and morphology across geographic regions (Madec, Bellido & Guiller 2003) and shows physiological and morphological adaptations associated with dry, cold and hot environments (e.g. metabolic depression, reduction of water-loss rate, thicker shell, reduced aperture of the shell, thicker epiphragm and supercooling ability) (Bishop & Brand 2000; Ansart & Vernon 2004). Additionally, empirical evidence has shown that terrestrial gastropods are energetically constrained by their mode of locomotion (Denny 1980)

and the cost of shell production during ontogeny (Czarnoleski *et al.* 2008).

MAINTENANCE AND HUSBANDRY OF ANIMALS

Two hundred adult snails (mean \pm SD; 4.21 ± 0.63 g, approximate age: <1 year) (Madec, Desbuquois & Coutellec-Vreto 2000) were collected by hand from gardens and parks in each one of the three localities ($N = 600$), placed in plastic containers and transferred to the laboratory at the Universidad Austral de Chile in Valdivia. According to their original populations, animals were individually marked with shell numbers and housed in big plastic cages ($60 \times 60 \times 13$ cm) filled with 10 cm of humid soil. The snails were maintained at densities of 50 animals per cage and were fed *ad libitum* with a mix of corn-wheat flour and calcium carbonate (1 : 1 : 0.3). We used four big cages per population (no crosses between populations were allowed, see details in Nespolo *et al.* 2014). The temperature and photoperiod for rearing conditions were 20 °C and 16 : 8 L : D. Water availability was maintained at high levels by sprinkling the interior of the cages with water daily. From these cages ('F₀' individuals), we collected entire clutches, individuals from which were grown in isolation to produce virgin individuals ('F₁' individuals) that were used to build the half-sib families. Clutches were maintained together until the juveniles were 2 months old (*c.* 100 mg). They were then individually located in small plastic cages of 100 mL, provided with litter, water and food *ad libitum*. We monitored adulthood by detecting the evagination of the peristoma (a sign of sexual maturity) (Madec, Desbuquois & Coutellec-Vreto 2000). Once adulthood was reached, we created each family by randomly designating a donor individual and four receptor individuals per family, since this species is a simultaneous hermaphrodite in which fertilization is reciprocal (Chase & Blanchard 2006). Each donor spent 1 week with each receptor, and then, it was switched to the next receptor. Two weeks between matings were allowed to let donors rest, and eggs produced by every donor were discarded. In all cases, as soon as oviposition was detected, the complete clutch (i.e. 'F₂' individuals) was removed from the litter, split in two and each half placed in two separate small cages. At this time, ten eggs were haphazardly collected and weighed with a microbalance (Radwag model MXA S/1; 0.000001 g). Snails were individually marked as soon as a hard shell appeared at 1 month of age and were kept in these cages until they were 3 months old. Then, each snail was isolated in a small cage until physiological measurements were taken at 5 months of age. In the following sections, we describe trait measurement according to the sequence (and meaning) presented in Fig. 1.

DIGESTION: FOOD CONSUMPTION AND DIGESTIBLE ENERGY INTAKE

After respirometric trials (see below), snails were maintained for 5 days in laboratory conditions with a diet of 100% lettuce to eliminate any intestinal remnants of the previous diet (flour mix). This diet change provoked a change in faeces colour from white to black, from which we could ascertain the changeover in diet. Animals were then deprived of food for 2 days and, starting on the third day, were provided with a known amount of dry wheat flour during 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 weight (± 0.0001 g). At the end of the experiment, apparent dry-matter digestibility (AMD) was calculated as $(I - E)/I$, where I is the food intake in grams and E is the egestion in grams. We call this variable 'apparent' because endogenous protein and gut microflora could contribute to faecal mass, but cannot be measured with this approach (Karasov & Martinez del Rio 2007). To calculate energy

digestibility, the energy content of food and faeces were determined in a Parr 1261 computerized calorimeter (Parr Instrument Company, Moline, IL, USA). However, there was no difference in the faecal energy content among populations. For this reason, we only considered AMD.

MORPHOLOGY: SHELL DARKNESS AND BODY MASS

The snail's shell darkness (ShD) was estimated using digital images in 8 bits greyscale, where 0 is black and 255 is white. The pictures were taken in a dark room with the camera mounted on a tripod, using the same focal distance and luminance conditions. The ShD was computed with IMAGE J[®] software (Wayne Rasband, NIH, Bethesda, MD, USA) where the ShD value was the mean pixel value in a circumference drawn on the image of shell's dorsal view. To establish snail's body size, its body mass at the nearest 0.01 g was estimated. This trait was measured during the week previous to the beginning of the selection experiment.

MAINTENANCE COSTS: STANDARD 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 using an open system, as described by Gaitan-Espitia *et al.* (2013). In brief, CO₂ production at 20 °C was measured continuously with an infrared CO₂ analyzer (LI-COR 7000 Sable System[®]; Li-Cor, Lincoln, NE, USA) capable of detecting differences of 1 part per million (p.p.m.) of CO₂ in air, connected to a computerized data acquisition system (EXPE DATA software; Sable Systems, Las Vegas, NV, USA, see Lighton 2008). The analyzer was calibrated periodically against two kinds of gas (CO₂-free air, and a commercial mix of 291 p.p.m. of CO₂). A Sable System eight-channel multiplexer was used to perform the measurements, five chambers with individual snails and three chambers for baseline measurements (before and after all records), which allowed for correction of possible drift (although it was almost non-existent between baselines).

The arrangement of the respirometry system was as follows. To remove water vapour and CO₂, ambient air was first pumped at 100 mL min⁻¹ through a Drierite/soda lime column. The air was then passed through a single flowmeter maintaining a constant ($\pm 1\%$) flow rate through the respirometric chambers. CO₂-free air was always flowing through all chambers while an auxiliary pump was measuring one of them. We used transparent metabolic chambers (60 mL), each one with a 100% hydrated snail. The temperature of the chamber was also continuously recorded with a copper-constantan thermocouple connected to the computer. Animal activity was visually monitored at intervals of *c.* 10 min during measurements, which lasted over 60 min each. Activity was rarely observed during the respirometry measurements, and the data of active animals were not included in the analysis. Data were recorded using LI7000 software and were imported as a text file into EXPE DATA (v 10.019; Sable Systems International). Data of CO₂ in p.p.m. were converted to mL h⁻¹ by a macro program in the EXPE DATA software (Sable Systems) taking into account the flow rate, using the equation:

$$VCO_2 = STP \times (FeCO_2 - FiCO_2) \times FR / (1 - FeCO_2 \times (1 - (1/RQ)))$$

where VCO₂ is carbon dioxide production, FeCO₂ is the excurrent fractional concentration of CO₂, FiCO₂ is the input fractional concentration of CO₂, FR 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 (=respiratory quotient). We assumed RQ = 0.85 (in this equation, large variations in RQ provoke differences in VCO₂ of <3%). Every trial had a minimum duration of 1 h and ranged from 60 to 75 min. In most cases, snails rested attached to the wall of the chamber during the whole recording (we repeated a few cases when individuals were active). To calculate VCO₂ with the above equation, we took the average of the last 30 min of recording. In order to achieve a post-absorptive state, metabolic rate was measured in 18-h food-deprived individuals (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 (pers. obs.). All individuals were weighed at the beginning, and at the end of the test period, the mean body mass (M_B) was recorded. The difference between initial and final mass was used as a proxy of body water loss (dehydration).

GROWTH: GROWTH RATE

To estimate the GR, we used mass increment (g day⁻¹) measured between the respirometry trials (before every trial, in order to avoid any error because of dehydration) and the beginning of digestive performance trials (1–1.5 months), at the nearest 0.01 g. By this procedure, we assumed a linear growth.

SURVIVAL: PHENOTYPIC SELECTION EXPERIMENT

After trait measurements, approximately 1400 snails, raised in a common garden design and individually marked, were available (450 per original population, hereafter 'source population'; Table 1). At the beginning of the summer (January), half of the snails were released in La Serena (North) and the other half in Valdivia (South) (hereafter 'location'). In each location, six timbered boxes or ('enclosures', hereafter) of 1.5 m (height) \times 2 \times 2 m that provided 4 m² of ground surface and an inner surface of 16 m² (including walls) were installed. Enclosures were arranged in a rectangular manner (2 \times 3), at the main campuses of Universidad de La Serena (La Serena) and Universidad Austral de Chile (Valdivia), and arranged as the conditions these garden snails experience normally. At each location, enclosures were surrounded by a rectangular fence of 2 m (height) 10 \times 15.5 m to avoid disturbances. In each enclosure, we put a removable cap provided with a plastic net of 2 mm aperture to avoid predation. In order to mimic the density and habitat of the original populations, we set a density of *c.* 30 snails per m² (6 snails per m² of inner surface, which includes the walls of the enclosures) and placed stones on the ground surrounded by typical garden plants. In total, we released *c.* 120 snails in each enclosure (Table 1). Each experimental enclosure was exhaustively examined monthly to collect dead snails (empty shells), during 1 year. When the experiment ended, all snails were captured (dead and alive) and the complete cover of plants and grass was removed to assure collecting all shell leftovers. By this procedure, we were capable to collect and identify unambiguously all dead individuals due to the tagged empty shells. In summary, we had snails from three populations (La Serena, Constitución and Valdivia) that passed two generation in the laboratory and then they were released in two locations (La Serena and Valdivia), and in each location, they were distributed in six enclosures, ordered in two enclosures per population (snails from different populations were not mixed in a given enclosure).

Table 1. Traits (abbreviations and units in parenthesis), sample sizes and means (\pm SEM) in three Chilean populations of the land snail *Cornu aspersum* across a latitudinal gradient of 1300 km. Analyses of variances showed significant differences between populations for almost all traits except in residual standard metabolic rate (SMR_R) and growth rate (GR)

Traits	La Serena (30°LS)	Constitución (36°LS)	Valdivia (39°LS)	ANOVA
	Mean \pm SE	Mean \pm SE	Mean \pm SE	
Body size (M_B , g)	1.877 \pm 0.039 (a)	1.754 \pm 0.035 (a)	2.133 \pm 0.045 (b)	$F_{2,1347} = 22.32$; $P = 0.0001$
Shell darkness (ShD, greyscale colour index)	36.133 \pm 0.447 (a)	39.993 \pm 0.457 (b)	39.782 \pm 0.530 (b)	$F_{2,1347} = 21.41$; $P = 0.0001$
Residual standard metabolic rate (SMR_R , mL CO ₂ h ⁻¹)	-0.008 \pm 0.003 (a)	-0.002 \pm 0.02 (a)	-0.001 \pm 0.003 (a)	$F_{2,1347} = 3.288$; $P = 0.376$
Absolute standard metabolic rate (SMR, mL CO ₂ h ⁻¹)	0.096 \pm 0.003 (a)	0.085 \pm 0.02 (b)	0.101 \pm 0.003 (a)	$F_{2,1347} = 9.76$; $P \leq 0.001$
Apparent dry-matter digestibility (AMD, g)	0.475 \pm 0.008 (a)	0.499 \pm 0.009 (a)	0.441 \pm 0.009 (b)	$F_{2,1347} = 10.46$; $P = 0.0001$
GR (GR, g day ⁻¹)	0.016 \pm 0.001 (a)	0.014 \pm 0.001 (a)	0.016 \pm 0.001 (a)	$F_{2,1347} = 1.587$; $P = 0.205$
Sample size	($N = 474$)	($N = 469$)	($N = 407$)	

Different lowercase letters in parentheses indicate significant differences between populations (Tukey multiple comparisons of means, 95% familywise confidence level).

STATISTICS

We measured viability selection by computing longevity as survival surrogate (our fitness estimate) as the number of months a snail was seen alive, which ranged from 1 to 12 (McGlothlin *et al.* 2005). We used linear regression models to estimate linear, nonlinear and correlational selection gradients (Lande & Arnold 1983; Brodie & Janzen 1996) separately for the two experimental sites. All analyses were performed with the 'r' software version 2.10 (<http://www.r-project.org/>). Prior to analyses, traits were standardized to mean = 0 and SD = 1. Fitness measurements (months of survival) were standardized to relative fitness (dividing each datum by the mean fitness of the sample). We used the statistical package *lm* (linear models) (Crawley 2007) to detect fitness (survival) effects of the phenotype, and the surface of selection (i.e. shape) was estimated by cubic splines also on standardized traits, using the *fields* statistical package. Because our original measure of fitness was non-normally distributed and could not be transformed to fit normality, the data were fitted by a generalized linear model (GLM) with gamma distribution to test the significance of the parameters (Christie *et al.* 2012). The selection gradients were estimated with linear models (LM); first, with the linear terms to obtain the directional coefficients, and secondly, with all terms (linear, quadratic and cross-product) to obtain the quadratic and correlational coefficient. Although many authors have used the direct estimation of selection coefficients, according to Stinchcombe *et al.* (2008), this practice has underestimated the strength of nonlinear selection. Given that the quadratic selection coefficients directly obtained from the statistical analysis are halved, it needs to be multiplied by two (Stinchcombe *et al.* 2008).

We performed separate analyses per location, and we started the analysis building a full model that included *population* (site of origin) and the following variables: standard metabolic rate (residuals of linear regression with body mass, SMR_R), GR, ShD, body mass (M_B) and the quadratic term of each variable. In these initial models, we also evaluated the following interactions as pair of traits: $SMR_R \times GR$, $SMR_R \times ShD$, $SMR_R \times BM$, $GR \times ShD$, $GR \times BS$ and $ShD \times BM$. We selected a minimum adequate model with a backward stepwise procedure using the exact Akaike's information criterion, with the step function implemented in the R package 'stats' version 3.0. The model selection procedure started with a full model and was automatically finished when the difference in the Akaike Information Criterion (AIC) values between two models was (arbitrarily) < 2 . Data to test survival differences between populations in the two experimental sites were

analysed with the *survival* R statistical package. We used a parametric survival fit model (Weibull distribution) for each experimental site and log-rank test for the effects of population.

Results

The means comparison showed that some of the traits were significantly different among localities (Table 1). In the case of absolute standard metabolic rate (SMR), this appears to be the effect of differences in body mass (M_B) as residual SMR (SMR_R) does not show differentiation (Table 1). Survival curves showed that the proportion of surviving individuals at both locations was reduced across time (Fig. 2). However, the experiment at La Serena revealed that survival varied among source populations (Fig. 2a; $\chi^2_2 = 18.3$; $P < 0.001$; log-rank test). At Valdivia, however, we did not find a significant interaction ($\chi^2_2 = 3.0$; $P = 0.227$; log-rank test), indicating that all three populations performed similarly.

In terms of phenotypic selection, we measured a number of traits with several possible interactions (see Fig. 1). However, we only detected selection on three traits, with no effects of location or source population. Also, we did not find any significant interaction among these factors. Regarding the five traits we measured, which encompass morphological and functional traits (see Fig. 1), we did not detect any statistical effect of the enclosures (i.e. either of the two replications per population per location), location (i.e. La Serena vs. Valdivia) or source population (i.e. La Serena, Constitución or Valdivia). The only trait that showed similar selection regimes at both locations (stabilizing selection) was SMR_R (Table 2). Moreover, after testing all possible pairs of traits and using the stepwise procedure to select the minimum adequate model, only residuals of SMR (SMR_R ; the absolute rates of metabolism did not show different effects, see Appendix 1) and body mass (M_B) showed significant selection coefficients in both locations (Table 2). Growth rate showed directional selection

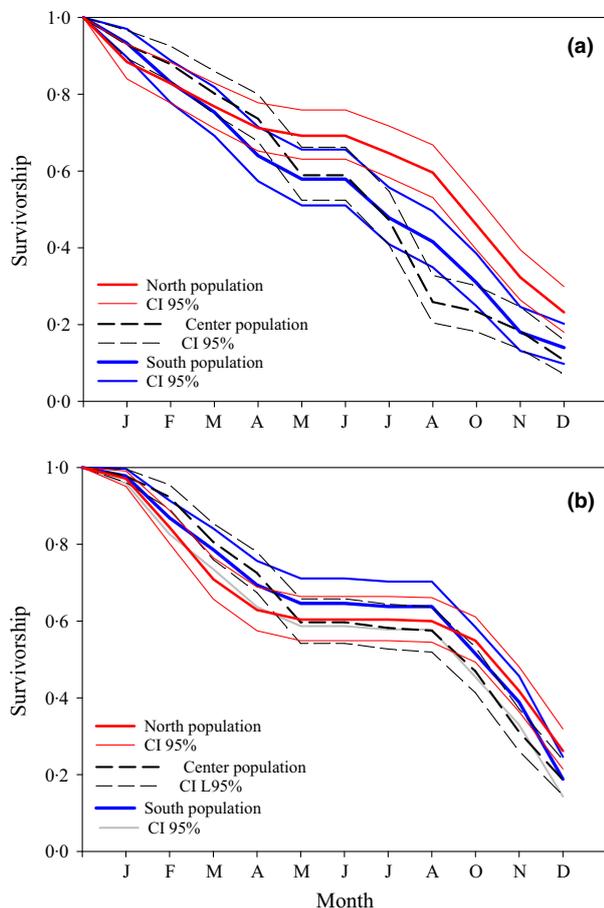


Fig. 2. Survival curves for snails from the three studied populations across a latitudinal gradient. Red: La Serena (north); black-dashed: Constitución (centre) and blue: Valdivia (south). Snails were raised at either extreme of the geographic gradient: La Serena (a: North) and Valdivia (b: South). Only in the north extreme (La Serena), we found interaction between the populations of origin ($\chi^2_2 = 18.3$; $P < 0.001$) but not in Valdivia ($\chi^2_2 = 3.0$; $P = 0.227$) after a log-rank test (Schimpf, Matthews & White 2012).

only in Valdivia (Table 2). We did not find that acquisition processes (i.e. digestion rate, here measured as apparent matter digestibility) or morphology other than M_B (i.e. darkness; see Fig. 1) were targets of natural selection across the geographic range of *C. aspersum*.

Given that the effects of enclosure, population and location were not significant; here, we present the analysis of selection using pooled data (Table 2). In La Serena, we found a significant negative quadratic coefficient both in SMR_R and M_B , indicating stabilizing selection (Fig. 3). In Valdivia, however, we only found directional selection on M_B (Table 2; Fig. 4) and GRate, and stabilizing selection on SMR_R (Table 2; Fig. 4). Interestingly, the selection differentials estimated in a monthly basis showed important differences between locations (Fig. 5). In La Serena, selection differential in SMR_R and M_B was maintained close to zero most of the year, with a positive peak in September that is cancelled by a sharp reduction below zero in

October (Fig. 5). On the contrary, at Valdivia, there was a monotonic increase in selection differentials towards the end of the year (Fig. 4).

We found negative correlational selection on the combination of SMR_R and M_B in La Serena (Table 2). This means that high values of SMR_R combined with small body sizes (or vice versa) were promoted (Fig. 6).

Discussion

Organisms often respond to differences in environmental conditions showing local adaptation in functional traits. This is the case for a number of invertebrates such as *Drosophila* (desiccation resistance) (Kellermann *et al.* 2009), amphipods (oxygen uptake) (Whiteley *et al.* 2011) and woodlice (energy metabolism) (Lardies, Bacigalupe & Bozinovic 2004). In this study, we measured a range of trait and functional capacities in an invasive species over its colonized range, using a common garden experiment combined with a reciprocal transplant.

In general, we found that individuals from the northern population appear to be performing better in its home location (compared with snails from the other locations), but no differences existed between snails when raised at the southern location, a result that does not fulfil the criterion to conclude local adaptation (Kawecki & Ebert 2004). Moreover, in both locations, residual standard metabolic rate (SMR_R) and body mass (M_B) were targets of selection. In fact, both in La Serena and Valdivia, selection promoted average SMR_R (i.e. stabilizing selection); but in M_B , there were different outcomes (i.e. stabilizing selection in La Serena and positive directional selection in Valdivia). Interestingly, in La Serena, we found negative correlational selection between SMR_R and M_B that judging from the graphics seems to be selection mainly favouring high metabolism and large sizes; large snails with low metabolism and small snails with very high metabolism.

One of the problems with correlational selection, which is difficult to detect, and in most cases has been documented for combinations of behaviour and morphology (McGlathlin *et al.* 2005; Peiman & Robinson 2012; Hahn & Muller-Scharer 2013), is the fact that it is also often difficult to interpret (especially when it is negative). Negative correlational selection means that opposite values of the trait maximize fitness. In our case, negative correlational selection was detected at both locations (although was significant only at the north), which would suggest that large snails have higher survival because of their size, finding refuge within themselves to save water, especially at the dry, warm and less productive climate of the north. Small individuals, on the contrary, need to be moving to find food and water and compensate their comparatively higher desiccation rate.

Another somewhat puzzling result was directional selection on GR only in the south. The hypothesis of counter-gradient variation in GR proposes that ectotherms from populations at high latitudes tend to grow quicker than

Table 2. Standardized directional (β_i), quadratic (γ_i) and correlational (γ_{ij}) selection gradients obtained from the best model chosen by Akaike Information Criterion (AIC) criteria in the two experimental sites (La Serena and Valdivia) with pooled populations data. The traits were standardized to mean = 0 and standard deviation = 1, and the relative fitness was obtained dividing the individual fitness (month survival that ranged between 1 and 12) by the mean of the sample. At each location (La Serena or Valdivia), the best model was searched using the AIC criterion (see Materials and methods for details). Then, the model was fitted by mean of a generalized linear model (GLM) procedure to test the significance of the parameters. The selection gradients were estimated performing linear models (LM), first, with the linear terms to obtain the directional coefficients, and secondly, with all terms (linear, quadratic and cross-product) to obtain the quadratic and correlational coefficients. The selection gradients estimates are presented with \pm the standard error

Trait	La Serena (30°)		Valdivia (39°)	
	Estimates \pm SE	<i>F</i> -value _(d.f.)	Estimates \pm SE	<i>F</i> -value _(d.f.)
SMR _R (β_i)	0.007 \pm 0.019	0.128 _(1,571)	0.005 \pm 0.014	0.010 _(1,775)
ShD (β_i)	-0.022 \pm 0.019	1.273 _(1,570)	-0.022 \pm 0.014	1.303 _(1,774)
Body mass (β_i)	-0.022 \pm 0.019	0.381 _(1,569)	0.037 \pm 0.014	13.556 _(1,773) ***
GRate (β_i)			0.041 \pm 0.015	6.828 _(1,772) **
SMR _R (γ_i)	-0.022 \pm 0.008	4.111 _(1,568) *	-0.024 \pm 0.005	10.267 _(1,771) **
Body mass (γ_i)	-0.110 \pm 0.014	20.441 _(1,567) ***	-0.042 \pm 0.009	3.012 _(1,770)
SMR _R \times ShD (γ_{ij})	-0.044 \pm 0.018	2.977 _(1,566) *		
SMR _R \times Body mass (γ_{ij})	-0.042 \pm 0.015	4.539 _(1,565) *	-0.024 \pm 0.010	2.049 _(1,769)
ShD \times Body mass (γ_{ij})			0.052 \pm 0.013	2.762 _(1,768)
ShD \times GRate (γ_{ij})			-0.062 \pm 0.019	2.232 _(1,767)
Sample size	(N = 573)		(N = 777)	

SMR_R, residual standard metabolic rate; ShD, shell darkness; GRate, growth rate. Empty spaces indicate that the trait was not selected in the best model in this experimental site. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

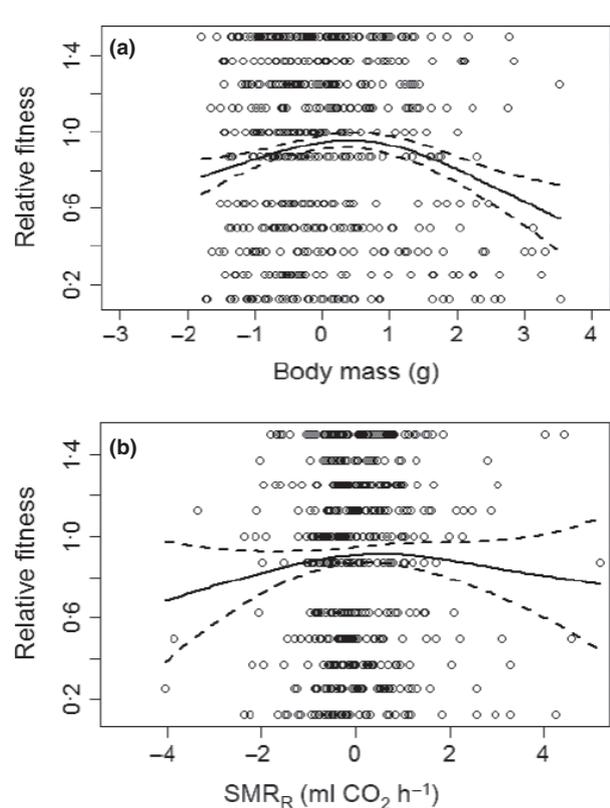


Fig. 3. Empirical fitness (survival) surfaces for the selection gradient are presented in Table 2, in La Serena, showing stabilizing selection on body mass (a) and on residuals of standard metabolic rate (b). Coefficients were obtained after an Akaike Information Criterion selection protocol including all traits and their paired combinations. Traits are standardized for mean = 0 and SD = 1 (see text for details).

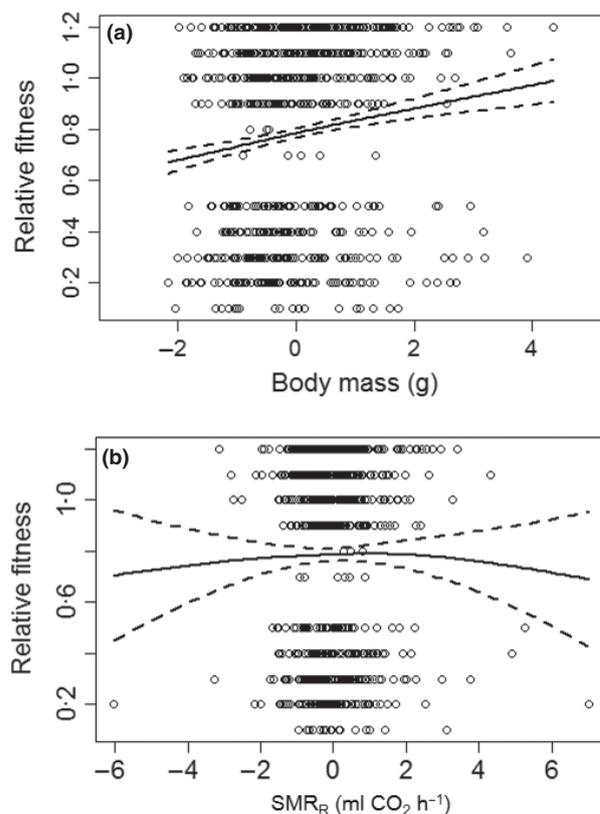


Fig. 4. Empirical fitness (survival) surfaces for the selection gradient are presented in Table 2, in Valdivia, showing directional selection on body mass (a) and stabilizing selection on residuals of standard metabolic rate (b). Coefficients were obtained after an Akaike Information Criterion selection protocol including all traits and their paired combinations. Traits are standardized for mean = 0 and SD = 1 (see text for details).

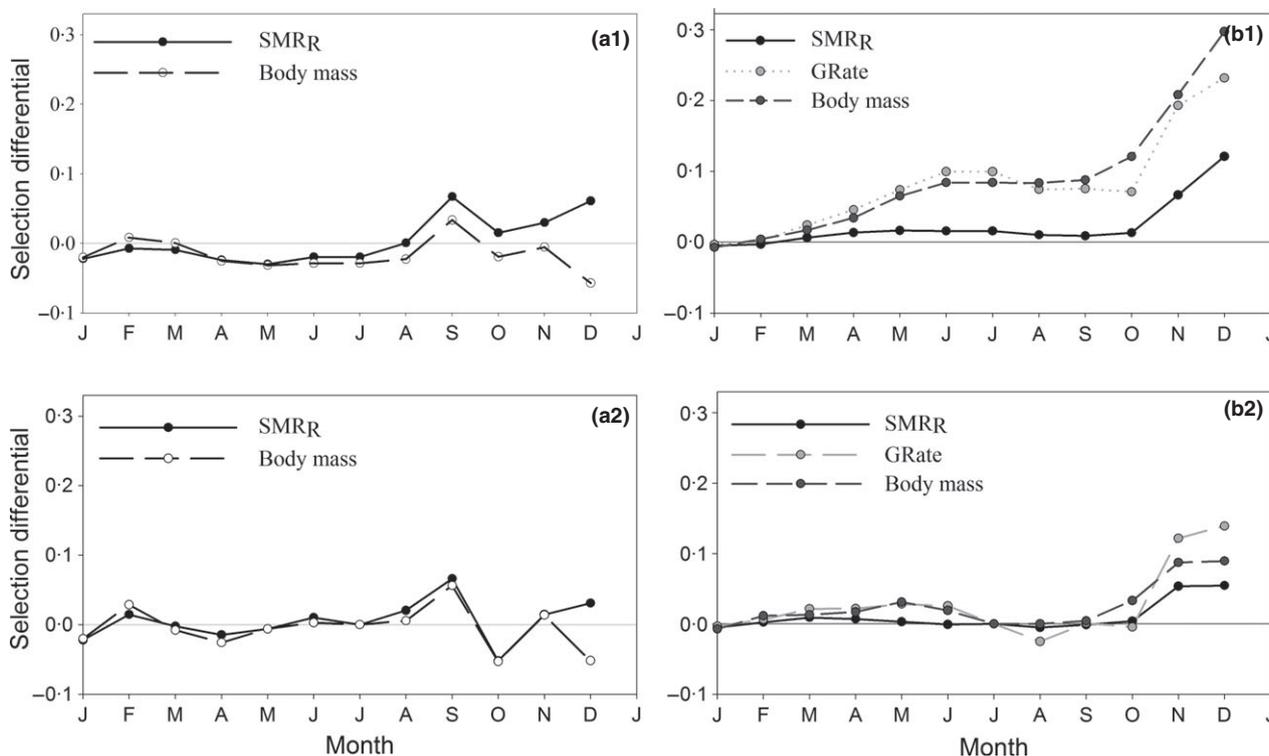


Fig. 5. Temporal variation directional of selection differentials (i.e. positive values indicate selection promoting an increase in the state of the character) in standardized traits that were under selection in each experimental location (a ‘La Serena’, b ‘Valdivia’). The upper graphs (a1, b1) show selection differentials estimated considering the time progress (t1–t0), (t2–t0), (t3–t0)...(t12–t0), and the lower graphs (a2, b2) show selection differentials monthly estimated (t1–t0), (t2–t1), (t3–t2)...(t12–t11). SMRR (residual standard metabolic rate), GRate (growth rate).

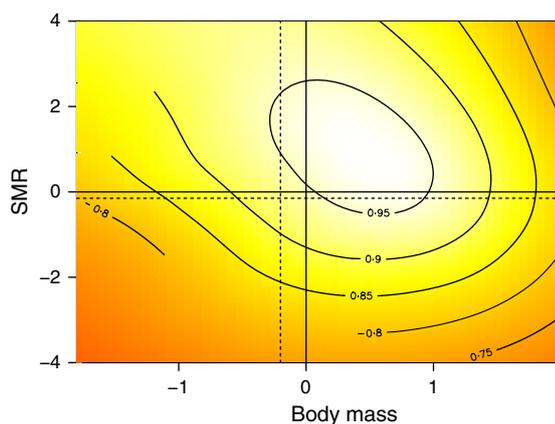


Fig. 6. Fitness contour (individual probability of survival; 0–1) showing the joint effects of SMRR and body mass on fitness. A single fitness peak (i.e. values above 0.95 survival probability) is shown by the clearest spot at the center. Traits are standardized for mean = 0 and SD = 1 (see text for details). The dotted line shows the median for each trait and the symbols represent the means of each population: triangle = La Serena, circle = Constitución and square = Valdivia.

those of low latitudes, as a way to compensate for the short season (Conover & Schultz 1995; Niewiarowski & Angilletta 2008). It is interesting to note that *C. aspersum* snails appear to follow this pattern (Diego Gaitan-Espitia & Nespolo 2014), which is corroborated with our results,

as higher GRs, body sizes and SMRR (late in the season) were promoted in the south (Bruning *et al.* 2013; Nespolo *et al.* 2014). This result, together with the fact that we did not find evidences of local adaptation, indicates that south imposes some severity to the reproductive schedule of these snails because of the short season.

In a previous study, negative directional selection on SMRR, combined with stabilizing selection, was found in *C. aspersum* at Valdivia (Artacho & Nespolo 2009). That is, individuals with average-to-low SMRR had higher survival probabilities. Artacho & Nespolo (2009) explain this result in the context of the allocation principle, suggesting that austere individuals had a surplus of energy that reduced mortality. However, an alternative explanation is related to a correlated response of (negative) selection on activity. In fact, in several insect species, the ‘freeze’ anti-predator behaviour represents an important survival strategy, provoking that more active individuals are selected against; consequently, a correlated response on resting metabolic rate is detected (Krams *et al.* 2013). For the case of snails studied by Artacho & Nespolo (2009), an obscured effect of predation is possible, as more active snails could have been more conspicuous for predators (birds and rats, mostly). This net effect of selection on activity and metabolism is also explained by the ‘pace of life’ syndrome, according to which more active individuals invest proportionally more in early reproduction, with a

cost in longevity (Careau *et al.* 2010). This is because oxidative damage is increased in individuals with high levels of activity (and metabolism), then generating a trade-off between reproductive output and survival (Fletcher *et al.* 2013). However, this study was performed with higher statistical power (777 individuals at Valdivia, compared with 285 in Artacho & Nespolo 2009) and excluding predation effects. These contrasting results between two studies on the same species warrant further exploration, and points to predation as an important factor to be considered.

Studies of selection combined with common garden experiments are not abundant (especially with animals), and those performed with plants have shown important differences in magnitudes and shape of selection coefficients with latitude (Etterson 2004a,b), or in time (Siepielski, DiBattista & Carlson 2009). In our case, considering the number of possible effects and traits that we analysed in our snails, we expected several alternative selective scenarios. Comparatively warmer environments (i.e. La Serena, or 'north') may promote an increase in digestive efficiency, thus leading to positive directional selection on attributes related to nutrient uptake (e.g. Illius *et al.* 1995a; Pfaender, Schlieven & Herder 2010). Alternatively, colder environments (i.e. Valdivia or 'south') could promote conservative strategies of resource use that minimize energy expenditure, resulting in negative directional selection on SMR (e.g. Bochdansky *et al.* 2005; Artacho & Nespolo 2009). At the same time, the reciprocal translocation of source populations from either extreme could have evidenced complex interactions between populations and location (i.e. G \times E interactions). However, we found only that SMR_R (=maintenance costs) and body size were targets of selection in both extremes of the geographic range (GR was promoted by selection only in the southern location). Historically, both variables (i.e. body size and energy metabolism) have been considered essential aspects of organism performance, because they represent the amount of available tissue and energy for work. Body size is often considered the most important target of natural selection (Kingsolver *et al.* 2001; Scheihing *et al.* 2011) and/or a key optimizing criterion in multispecies analyses (Brown, Marquet & Taper 1993; West, Brown & Enquist 1997). On the other hand, the many versions of metabolic rate measurements such as standard metabolic rate, resting metabolic rate and maximum metabolic rate (see Lighton 2008) have been related to body size with different scaling exponents (Lehmann, Dickinson & Staunton 2000; Glazier 2005; Clarke, Rothery & Isaac 2010; Bruning *et al.* 2013). This reflects the fact that more tissue represents more energy consumption, a relationship that varies depending on the tissue involved, the organism and the environment. Several studies have shown selection on metabolic rates, either because they represent costs that need to be minimized (Marshall & McQuaid 2011; Careau *et al.* 2013) or capacities that enhance fitness (Moiroux *et al.* 2012; Schimpf, Matthews & White 2012; White, Alton & Frappell 2012). However, few times this has been empirically tested

together with other theoretically important variables, such as energy acquisition rates, growth or morphology (Sibly & Calow 1986; Mousseau & Roff 1987; Karasov 1992; Karasov & Martinez del Rio 2007). Moreover, in very few cases, this was performed under a common garden and reciprocal transplant. Here, we provide such a case, which we hope will inspire further experimental tests of local adaptation in functional traits in animal populations.

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Data accessibility

Data for this article are deposited in the Dryad Digital Repository: <http://doi.org/10.5061/dryad.n2677> (Bartheld *et al.* 2015).

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Appendix 1 Analyses using SMR as absolute values

Table A1. Standardized directional (β_i), quadratic (γ_i) and correlational (γ_{ij}) selection gradients obtained from the best model chosen by Akaike Information Criterion criteria in the two experimental sites (La Serena and Valdivia) with pooled population data. The traits were standardized to mean = 0 and standard deviation = 1, and the relative fitness was obtained dividing the individual fitness (month survival that ranged between 1 and 12) by the mean of the sample. The model was fitted by mean of a generalized linear model (GLM) procedure to test the significance of the parameters. The selection gradients were estimated performing linear models (LM), first, with the linear terms to obtain the directional coefficients, and secondly, with all terms (linear, quadratic and cross-product) to obtain the quadratic and correlational coefficients. The selection gradients estimates are presented with \pm the standard error. Empty spaces indicate a phenotypic trait that was not included in the best model in each experimental site

Trait	La Serena (29°)		Valdivia (39°)	
	Estimates \pm SE	<i>F</i> -value _(d.f.)	Estimates \pm SE	<i>F</i> -value _(d.f.)
SMR (β_i)	0.005 \pm 0.022	0.033 (1,571)	-0.004 \pm 0.015	0.733 (1,775)
ShD (β_i)	-0.020 \pm 0.019	1.411 (1,570)	0.021 \pm 0.014	1.353 (1,774)
Body mass (β_i)	-0.019 \pm 0.023	0.318 (1,569)	0.039 \pm 0.016	12.842 (1,773)***
GRate (β_i)	0.020 \pm 0.020	0.968 (1,568)	0.041 \pm 0.015	6.699 (1,772)**
SMR (γ_i)	-0.009 \pm 0.011	6.297 (1,567)*		
Body mass (γ_i)	-0.046 \pm 0.015	15.518 (1,566)***		
GRate (γ_i)	-0.008 \pm 0.007	0.484 (1,565)		
SMR \times ShD (γ_{ij})	-0.021 \pm 0.016	0.702 (1,564)	0.031 \pm 0.014	2.505 (1,771)
SMR \times Body mass (γ_{ij})			-0.031 \pm 0.010	17.680 (1,770)***
SMR \times GRate (γ_{ij})	-0.067 \pm 0.024	7.850 (1,563)		
Sample size	(<i>N</i> = 573)		(<i>N</i> = 777)	

SMR, standard metabolic rate; ShD, shell darkness; GRate, growth rate; SMR, standard metabolic rate.

P* < 0.05; *P* < 0.01; ****P* < 0.001.