

# Genetic variation for plasticity in physiological and life-history traits among populations of an invasive species, the terrestrial isopod *Porcellio laevis*

Marco A. Lardies<sup>1</sup> and Francisco Bozinovic<sup>2</sup>

<sup>1</sup>Departamento de Ciencias Básicas and Centro de Investigaciones en Ciencias Ambientales (CIENCIA-UST), Universidad Santo Tomás, Santiago and <sup>2</sup>Center for Advanced Studies in Ecology and Biodiversity (CASEB), Laboratorio Internacional de Cambio Global (LINC-Global) and Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile

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## ABSTRACT

**Background:** Evolutionary interpretations of life-history as well as physiological patterns require distinction between genotypic variations and environmentally induced phenotypic variation.

**Problem:** We investigate the extent to which variation in life history and metabolism have an environmental or a genetic basis in an invasive species.

**Methods:** We used the widely distributed isopod, *Porcellio laevis*, as a model. To examine the effects of latitudinal gradients in temperature and photoperiod on life-history and physiological traits, we studied three populations located along a latitudinal gradient. We performed two common garden experiments using 20 families from each population. Treatments were: (1) 12°C, 12/12 h light/dark and (2) 20°C, 14/10 h light/dark. Measurements of metabolic rate and life-history traits were performed on females from the  $F_1$  generation.

**Conclusions:** (1) Differences in life-history and metabolic traits among populations mimic the natural pattern of latitudinal variation in a cold environment, where offspring size and reproductive output increase towards higher latitudes and metabolic rate increases towards lower latitudes. (2) There is genetic variation for plasticity in response to the environment, which may be acted upon by natural selection. (3) Our results support the hypothesis that phenotypic plasticity itself is an adaptive response to environmental heterogeneity.

*Keywords:* common garden approach, geographic variation, micro-evolution, reaction norm, reproductive output.

## INTRODUCTION

Replicated latitudinal clines are of evolutionary interest because they provide evidence of the occurrence of natural selection (Endler, 1977; Gilchrist and Huey, 2004). However, geographic

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Correspondence: F. Bozinovic, Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile. e-mail: fbozinovic@bio.puc.cl

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variation itself is not proof of natural selection, since genetic drift and gene flow may also shape a pattern of clinal variation (Loeschcke *et al.*, 2000). Clinal variation in traits is often attributed to organismal adaptation to varying environmental conditions, especially due to temperature or temperature-related factors, which are considered to be the main agents of selection (Fox and Czesak, 2000; Ayrinhac *et al.* 2004; Kingsolver *et al.*, 2004).

Many morphological, life-history, and metabolic traits show signs of phenotypic plasticity (Schlichting and Pigliucci, 1998; Pigliucci and Preston, 2004). Furthermore, clinal variations in life-history and metabolic traits among populations are ubiquitous among ectotherms (see Blanckenhorn and Fairbairn, 1995; Gilchrist and Huey, 2004; Laugen *et al.*, 2005; David *et al.*, 2006). Indeed, intraspecific studies of geographic variation have provided convincing evidence for the occurrence of micro-evolution (Huey *et al.*, 2000; Miner *et al.*, 2005). Life-history traits are considered indirect measures of an organism's fitness (Stearns, 1992). Furthermore, physiological variation within the life history of an individual can have profound implications for fitness (Ricklefs and Wikelski, 2002; Lardies and Bozinovic, 2006). Since physiological maintenance costs are a large component of animal energy budgets (Sibly and Calow, 1986; Angilletta, 2001), geographic variation in life-history traits may be caused by differences in metabolic rates among individuals from different populations.

A single population can display annual or even seasonal variation in life-history and metabolic traits, and differences among a set of populations may be observed in some surveys but not in others; this is the result of phenotypic plasticity (Trexler *et al.*, 1990; Stearns, 1992; Roff, 2002). In this vein, how phenotypic plasticity evolves is controversial (Via *et al.*, 1995; De Jong, 2005), but all hypothesized mechanisms require genetic variation. Different effects of the environment on the phenotype of different genotypes result in variation in reaction norms, and a significant genotype  $\times$  environment interaction (Pigliucci, 2005). Genotype  $\times$  environment interactions are the type of genetic variation required for the evolution of phenotypic plasticity (Via and Lande, 1985; Miner *et al.*, 2005). Unfortunately, few studies have considered the change in phenotypic plasticity among populations along an environmental gradient, despite the fact that this type of study is ideal for understanding how phenotypic plasticity evolves.

The terrestrial isopod, *Porcellio laevis* (Isopoda, Oniscidea), offers an opportunity to examine variation in phenotypic plasticity of life-history and metabolic traits. Populations of this woodlouse occupy a wide latitudinal range and exhibit phenotypic variation in life-history as well as in physiological traits (Lardies *et al.*, 2004a, 2004b; Castañeda *et al.*, 2005; Lardies and Bozinovic, 2006). *Porcellio laevis* is an invasive species and is considered to be unusual among isopods given its ability to invade and establish viable populations in new habitats of widely different characteristics (Warburg *et al.*, 2001), and is one of the most broadly distributed species within the genus (Warburg, 1993). In this paper, we report the results of common garden experiments designed to investigate the extent to which variation in life history and metabolism have an environmental basis in an invasive species. Thus, we evaluated latitudinal variation in environmental factors as a source of variation in phenotype (phenotypic plasticity) and in the reaction norm. As pointed out by Huey *et al.* (2005), invasive species offer excellent opportunities to assess the rate and speed of evolution in the field as well as how they spread through new ecological scenarios.

## MATERIALS AND METHODS

### Study sites and breeding design

We collected isopods from three populations located along a latitudinal cline in Chile during spring/summer 2004. From north to south, the three populations were: Antofagasta (23°38'S), La Serena (29°55'S), and Santiago (33°23'S). Woodlice from all study sites were collected by hand from under stones, pieces of wood, and soil litter. Specimens were placed in containers with vegetable soil and carrot slices, and transported to the laboratory in Santiago. Climatological data for the different localities are presented in Table 1. Mean environmental temperature ( $T_a$ ) decreases gradually from northern towards southern Chile (Table 1). Total radiation is higher at the northern locality of Antofagasta and then decreases towards southern localities. Relative humidity is pretty constant, averaging approximately 76%. The pattern of precipitation is the inverse of that of total radiation, with least precipitation (~2.0 mm) in Antofagasta, with a gradual increase towards southern Chile (Table 1).

We performed two simultaneous common garden experiments in environmental chambers. In one experiment individuals were exposed to a cold environment ( $T_a = 12^\circ\text{C}$ ; 12/12 h light/dark), whereas in the other they were exposed to a warm environment ( $T_a = 20^\circ\text{C}$ ; 14/10 h light/dark). Each garden consisted of 20 families from each locality (60 families per garden), positioned randomly within each environment. Each family consisted of one male and two females in a Petri dish (50 mm diameter; with a base layer of plaster of Paris to maintain humidity), corresponding to a half-sib design (Roff, 1997). Food, in the form of dry spinach, and water were provided *ad libitum* in both environments. We placed females

**Table 1.** Summary of climatological variables for the three studied localities

	Antofagasta	La Serena	Santiago	Source
Latitude	23°38'S	29°55'S	33°23'S	
Longitude	70°26'W	71°15'W	70°42'W	
Altitude (m)	Sea level	Sea level	420	FAO (1985)
Mean annual temperature (°C)	17.0	14.9	13.9	FAO (1985); di Castri and Hayek (1976)
Mean minimum annual temperature (°C)	13.3	11.2	7.7	FAO (1985); di Castri and Hayek (1976)
Mean maximum annual temperature (°C)	20.1	18.9	22.1	FAO (1985); di Castri and Hayek (1976)
Mean annual rainfall (mm)	2.2 (0.0–3.5)	127.4 (55.2–185.3)	356.2 (84.0–700.2)	FAO (1985); di Castri and Hayek (1976)
Mean annual radiation (cal/cm <sup>2</sup> × d)	424	340	310	FAO (1985); di Castri and Hayek (1976)
Mean relative humidity (%)	72	80	75	FAO (1985); di Castri and Hayek (1976)
Climate	Desertic	Semi-arid	Mediterranean	di Castri and Hayek (1976)

and males together to allow for fertilization and egg extrusion by the females. Given that females can store sperm from several males (Moreau *et al.*, 2002), to ensure that fertilization occurred solely in the laboratory females from each locality were previously separated and isolated for at least 4 months under laboratory conditions that facilitate egg extrusion. From the second clutch obtained in the laboratory ( $F_1$  generation), we selected two female offspring from each mother and reared them under the same experimental conditions in separate containers for nearly 7 months of development. Metabolic and life-history trait measurements were conducted on adult females from the  $F_1$  generation. Once females from the  $F_1$  generation were fertilized by a male originating from their respective source population (egg extrusion was visible in the marsupium), the females were separated into individual incubation chambers until embryos developed into juveniles (mancae).

### Measurement of life-history traits

For each female we recorded offspring size and number. Furthermore, we estimated the reproductive output of each female following the equation presented by Clarke *et al.* (1991). Offspring were measured following emergence from the maternal marsupium. We measured offspring size as the total length of offspring (i.e. the distance between the mid-dorsal anterior margin of the carapace and the distal margin of the pleotelson). Females and their offspring were measured using a compound microscope equipped with a calibrated ocular micrometer. The body mass of each female was recorded using an analytical balance (CHYP JK-180) with a precision of  $\pm 0.01$  mg.

### Metabolic rate measurements

The body mass of each specimen was recorded using an analytical electronic balance (CHYO JK-180,  $\pm 0.0001$  g). We estimated the standard metabolic rate of each individual. For this measurement, carbon dioxide production ( $VCO_2$ ) was recorded in a computerized open-flow respirometry system (Sable Systems, Henderson, NV) in 10-ml metabolic chambers. Metabolic rate was measured after one week of acclimation at a mean environmental temperature of  $20.0 \pm 0.5^\circ\text{C}$ . We chose  $20^\circ\text{C}$  because it is close to the preferred temperature of this species (Castañeda *et al.*, 2004) and represents an appropriate 'portrait' of metabolic rate among populations (Lardies and Bozinovic, 2006). The metabolic chamber received dried air at a rate of  $150 \text{ ml} \cdot \text{min}^{-1}$  from mass flow controllers (Sierra Instruments, Monterrey, CA). The air was passed through  $\text{CO}_2$  absorbent granules (Baralyme) before entering the metabolic chamber. Carbon dioxide production was monitored four times per second over 30 min. Baseline measurements for each run were made using the same experimental chamber both at the beginning and at the end of each measurement period. Certified commercial gases of 0% and 5.3%  $\text{CO}_2$  were used as standards for calibration. Each record was automatically transformed by a macro program recorded in the Datacan software (Sable Systems, Henderson, NV). We estimated standard metabolic rate as the average of the 3–5 min steady-state  $VCO_2$  production during periods of inactivity (determined visually). Before and after each  $VCO_2$ , body mass was recorded using an analytical electronic balance ( $\pm 0.0001$  g), and the mean of both measurements was used for analysis. Woodlice were fasted for 2 days before the metabolic measurements.

### Statistical analyses

Phenotypic correlations among life-history and metabolic traits for each population and for each environment were estimated by Pearson product-moment correlations ( $r$ ). Mass-corrected  $V\text{CO}_2$  and offspring number values were computed and used for phenotypic correlations. A Bonferroni correction was applied to adjust significance levels for multiple comparisons.

The genotype  $\times$  environment ( $G \times E$ ) interaction in the life-history and metabolic traits was assessed as follows: the interaction term was computed to determine variation in  $G \times E$  in response to environment (cold or warm), according to the genotype upon which natural selection could operate (Via and Lande, 1985; Via, 1994). Interaction terms were computed for offspring size, metabolism, offspring number, and reproductive output. A mixed-model two-way analysis of covariance (ANCOVA) (Fry, 1992) was performed to determine the effects of genotype (source population) and environment (warm or cold) on offspring size, metabolism, and offspring number with female body mass as a covariate, using the General Linear Models option in the statistical software package Statistica (version 6.0; StatSoft Inc., Tulsa, OK). A mixed-model, two-way analysis of variance (ANOVA) was performed to determine the effects of genotype and environment on the reproductive output of females. The independent variables were (1) source population, designated as a random factor (assumed to be randomly selected from an infinite population of possible levels), and (2) environment, designated as the fixed factor (levels are predetermined). The  $G \times E$  term was also designated as a random factor. Replication between experiments was not equal since the reproductive success of females varied among families.

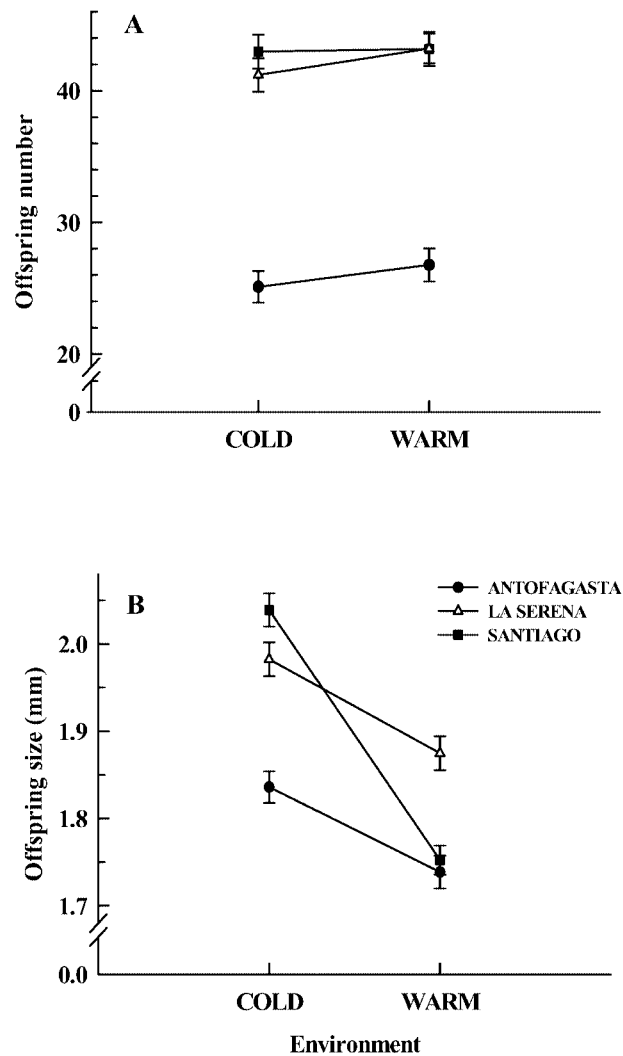
## RESULTS

### Life-history traits

Offspring number was significantly lower in individuals from Antofagasta than from La Serena and Santiago (Fig. 1, Table 2). Thus, source population had a significant effect on offspring number. Even when we used female body mass as a covariate, we continued to observe significant differences in offspring number among populations. A multiple comparison among means (Tukey's test,  $\alpha = 0.05$ ) revealed no significant differences in offspring number between the La Serena and Santiago populations. The combination of temperature and photoperiod did not have an overall effect on offspring number (Table 2).

Offspring size increased towards higher latitudes. This tendency was most evident in the cold environment. In addition, population origin had a significant effect on offspring size (ANCOVA,  $F_{2,273} = 23.24$ ;  $P = 0.0000$ ). The largest offspring were obtained from Santiago in the cold environment treatment. Tukey's test revealed significant differences ( $\alpha = 0.05$ ) in offspring size in a cold environment among all three populations, but in the warm environment significant differences were only observed between La Serena and the other two populations. Environment did not have a significant effect on offspring size (ANCOVA,  $F_{1,2} = 6.92$ ;  $P = 0.1192$ ).

In general, reproductive output decreased with decreasing latitude (Fig. 2). Population had a significant effect on reproductive output (Table 2), but was not significantly affected by the environment. The highest reproductive output was found in Santiago in the cold environment, while the lowest reproductive output was found in the Antofagasta



**Fig. 1.** Offspring number (A) and offspring size (B) in response to environment (cold or warm) in the three studied populations. Values for offspring size (mm) are mean sizes of each individual female  $\pm$  standard error.

population in the warm environment (Fig. 2). An *a posteriori* Tukey test detected significant differences between all populations in the cold environment, but only for the Antofagasta population under warm conditions.

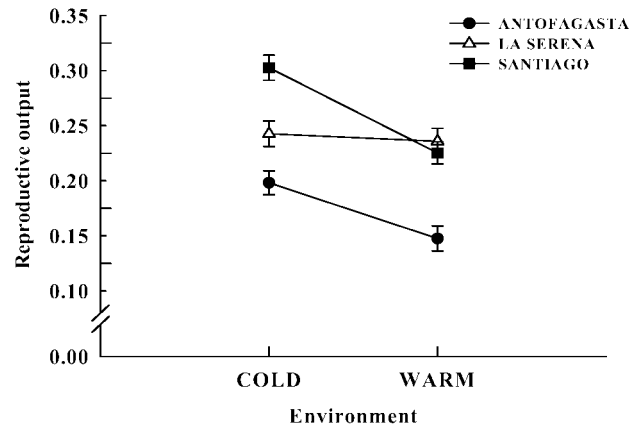
### Metabolic rate

Carbon dioxide production was significantly correlated with body mass, but was also affected by source population (Fig. 3, Table 2): metabolic rate in the  $F_1$  generation was significantly lower in the Santiago population than in the La Serena and Antofagasta

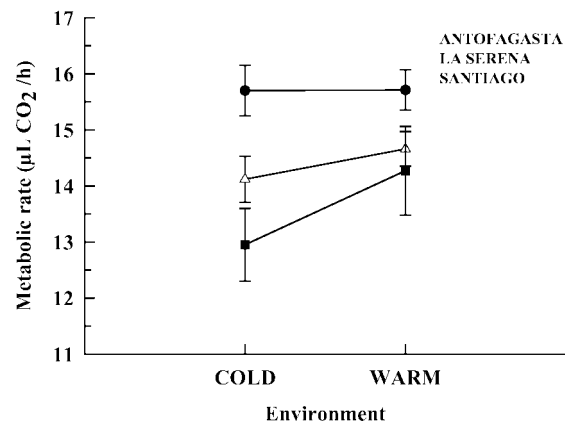
**Table 2.** Analyses of covariance (metabolic rate, offspring size and number) and reproductive output partitioning variance in life-history and metabolic traits into components due to population and environment

Offspring number			
Effect	d.f.	<i>F</i>	<i>P</i>
Population	2	17.84	0.0065
Environment	1	0.67	0.5016
Population × environment	2	25.78	0.0007
Error	476		
Offspring size			
Effect	d.f.	<i>F</i>	<i>P</i>
Population	2	55.00	0.0000
Environment	1	6.92	0.1192
Population × environment	2	135	0.0000
Error	476		
Reproductive output			
Effect	d.f.	<i>F</i>	<i>P</i>
Population	2	63.64	0.0000
Environment	1	4.67	0.1632
Population × environment	2	9.09	0.0042
Error	476		
Standard metabolic rate			
Effect	d.f.	<i>F</i>	<i>P</i>
Population	2	114.96	0.0001
Environment	1	5.99	0.1342
Population × environment	2	71.75	0.0001
Error	476		

*Note:* Population effects are random, environment effects are fixed. *F*-tests for environment effects use the mean square of population × environment interaction term as the error term.



**Fig. 2.** Reproductive output of females (F<sub>1</sub> generation) for the three studied populations exposed to two experimental environments (cold or warm). Values are means  $\pm$  standard errors.



**Fig. 3.** Metabolic rate of females (F<sub>1</sub> generation) for the three studied populations exposed to two experimental environments (cold or warm). Values are means  $\pm$  standard errors. See Methods for details.

populations. Even when we used female body mass as a covariate, we continued to observe significant differences in metabolic rate among populations. Tukey's test revealed significant differences ( $\alpha = 0.05$ ) in metabolism between populations of the F<sub>1</sub> generations from the La Serena and Antofagasta populations. Metabolic rate was significantly affected by environmental treatments (Table 2).

### Genotype $\times$ environment interactions

A significant G  $\times$  E interaction (population  $\times$  environment) contributed to the observed variance for all of the studied life-history and metabolic traits (Table 2). This interaction was evident from the intersecting reaction norms of the analysed traits (Figs. 1, 2, and 3).

The significant  $G \times E$  interaction for offspring size, number of offspring, reproductive output, and metabolic rate indicates the presence of genetic variation in plasticity in response to the environment.

### Phenotypic correlations

Examination of partial correlations among traits indicated significant, negative phenotypic correlations (trade-offs) between offspring size versus offspring number and reproductive output versus standard metabolic rate for families from Santiago in both environments ( $P < 0.05$ ; Table 3). Offspring size showed a significant, positive phenotypic correlation with reproductive output in the Santiago population exposed to warm conditions. The La Serena and Antofagasta populations did not exhibit significant phenotypic correlations among pairs of traits for either environment. We obtained large standard errors in the mean values of several correlations (Table 3), indicating a high degree of variability in the correlation coefficients, probably due to the relatively low number of families analysed (Roff, 1997).

**Table 3.** Phenotypic correlations (Pearson's product-moment correlations) between traits analysed in the different populations of the terrestrial isopod *Porcellio laevis*

<b>Antofagasta</b>				<b>WARM</b>
	Offspring size	Offspring number	RO	SMR
Offspring size	—	$-0.09 \pm 0.06$	$0.18 \pm 0.11$	$-0.06 \pm 0.04$
Offspring number	$-0.12 \pm 0.08$	—	$-0.11 \pm 0.04$	$0.11 \pm 0.06$
RO	$0.10 \pm 0.07$	$0.12 \pm 0.05$	—	$-0.13 \pm 0.09$
SMR	$-0.10 \pm 0.08$	$0.12 \pm 0.09$	$-0.14 \pm 0.08$	—
<b>COLD</b>				
<b>La Serena</b>				<b>WARM</b>
	Offspring size	Offspring number	RO	SMR
Offspring size	—	$-0.37 \pm 0.07^*$	$0.13 \pm 0.06$	$-0.10 \pm 0.08$
Offspring number	$-0.11 \pm 0.06$	—	$-0.05 \pm 0.04$	$0.09 \pm 0.06$
RO	$0.17 \pm 0.09$	$0.14 \pm 0.05$	—	$-0.13 \pm 0.06$
SMR	$-0.11 \pm 0.07$	$0.07 \pm 0.06$	$-0.13 \pm 0.07^*$	—
<b>COLD</b>				
<b>Santiago</b>				<b>WARM</b>
	Offspring size	Offspring number	RO	SMR
Offspring size	—	$-0.22 \pm 0.07^*$	$0.39 \pm 0.08^*$	$-0.08 \pm 0.06$
Offspring number	$-0.37 \pm 0.08^*$	—	$-0.10 \pm 0.06$	$0.11 \pm 0.06$
RO	$0.12 \pm 0.06$	$0.20 \pm 0.10$	—	$-0.25 \pm 0.09^*$
SMR	$-0.06 \pm 0.04$	$0.08 \pm 0.06$	$-0.33 \pm 0.08^*$	—
<b>COLD</b>				

\*Statistically significant differences within population correlations ( $P < 0.05$ ). RO = reproductive output, SMR = standard metabolic rate. Values are means  $\pm$  standard errors.

## DISCUSSION

### Latitudinal variation in life-history and metabolic traits

The environmental contribution of latitudinal differences in life-history traits was evident from the differential responses of individuals of the studied invasive species derived from the same population of origin, especially in Santiago (see also Warburg, 1993; David *et al.*, 2001; Hassall *et al.*, 2005). In the present study, variation in temperature and photoperiod yielded differential expressions of life-history traits in both mothers and offspring. Offspring size at release and/or egg size is related to the fitness of individual offspring in many arthropods (Lawlor, 1976; Brody and Lawlor, 1984; Fox and Czesak, 2000). The offspring size of *Porcellio laevis* increased toward high latitude, especially in the cold. Such temperature-induced plasticity appears to be widespread in ectotherms (Atkinson *et al.*, 2001). We also observed an increased number of eggs and higher reproductive output at higher latitudes. A similar pattern has been reported for these traits in *Drosophila melanogaster* – another invasive species – on more than one continent (Azevedo *et al.*, 1996; Van't Land *et al.*, 1999). Egg size is considered to be a good estimator of egg energy content in crustaceans (Clarke, 1993). Accordingly, *P. laevis* offspring from high latitudes are larger and contain more internal energy reserves, which may enable individuals to withstand continued environmental changes and short growing seasons in cold regions (Bauer, 1992; Blanckenhorn, 1997). As pointed out by Lawlor (1976), this phenotypic trait ensures that either large juveniles are produced, or that juveniles hatch with sufficient reserves to grow rapidly through the vulnerable size range. Indeed, the larger juvenile size in individuals from high latitudes may convey a high starvation tolerance and a greater resistance to fluctuations in environmental factors.

Latitudinal clines in ectotherms' traits are largely caused by the environment. The environment can have both short-term and long-term effects, ranging from an immediate developmental response to evolutionary adaptation over a number of generations. Seasonality in both food and temperature has been suggested to explain latitudinal clines in life-history traits (see Fox and Czesak, 2000; Lardies and Castilla, 2001; Ricklefs and Wikelski, 2002). However, the strongest candidate for a selective agent is temperature (Robinson and Partridge, 2001; Fischer *et al.*, 2004). Furthermore, in terrestrial isopods, temperature can have a significant effect on juvenile survival (Sutton and Holdich, 1984). We found that offspring number was consistently higher in warm environments. In addition, there were differences in offspring size and reproductive output between cold and warm environments, as well as between populations. The effects (common garden) found in our study parallel those observed in nature (Lardies and Bozinovic, 2006), implying that the clines we found are based on genetic differences between populations, and are probably the result of natural selection. Thus, thermal physiology may be a significant factor underlying the ecological and evolutionary success of animals, as well as the likely impact of climatic change.

The source population has also a significant effect on reproductive output, offspring size and number. Indeed, individuals raised in a cold environment mimicked the natural pattern more than individuals raised in a warm environment. The patterns of energy allocation were probably genetically altered, as indicated by the significant genotype  $\times$  environment interaction (see below). The patterns observed in the present study predict a decrease in reproductive output with increasing temperature. This result is likely related to the number of egg batches produced during the reproductive season and/or the number of days of embryonic development. In general, isopods in cold environments have slow gametogenesis

and longer gonadal and embryonic development times than individuals in warm environments (Warburg, 1987, 1993). Consequently, the quantity of egg batches produced during one reproductive season may be higher in individuals maintained in warm environments. This pattern also suggests that individuals in a warm environment concentrate more energy towards maintenance and growth, and invest less energy in reproduction (see Table 3). Inversely, in cold environments individuals have lower maintenance costs, which allow higher energy investment in egg production.

Since the size of an offspring and the number of offspring produced are usually opposing attributes, selection for one attribute will forfeit the other (Bernardo, 1996). Thus, if investment in offspring production is fixed and all else is equal, populations with large offspring should have small offspring numbers and vice versa. We observed that reproductive output in *P. laevis* increases towards high latitudes, which consequently increases offspring size and number. Also, given that the phenotype of an individual is a collection of interlinked traits, we expected a correlation among the different traits. Our results indicate a number of significant, positive and negative phenotypic correlations among traits (Table 3), but only for the Santiago population. In the F<sub>1</sub> generation we established the existence of trade-offs between offspring number and offspring size, as well as between reproductive output and metabolism. These trade-offs were found in both environments. When considering offspring number in equally sized ovigerous females of *P. laevis*, the number of offspring of these females increased from high to low latitudes. These differences can be explained by the fact that offspring size is negatively correlated with offspring number in many species (France, 1992; Fox and Czesak, 2000; Wilhelm and Schindler, 2000). Indeed, in our common garden experiments, the Santiago population showed great phenotypic variation in the analysed traits (Fig. 2). Females from Santiago are able to produce a large quantity of small offspring, or to produce a smaller quantity of large offspring. Theoretically, the costs of maintenance may have important effects on the quantity of energy available for activity and reproduction (Angilletta, 2001). Implicit in the covariation between reproductive output and maintenance cost is that between-population variations in standard metabolic rate result in higher fitness in individuals from different populations. The Santiago population showed high intra-population variation in standard metabolic rate and reproductive output of approximately 32% and 28%, respectively, which accounts for the significant, negative phenotypic correlation between these traits in both environments. We believe that intra-population differences in metabolic rate and reproductive output could be caused by different levels of activity and/or levels of energy expenditure associated with seasonal differences (in food availability) where they grow, develop, and reproduce. This suggests that some individuals at high latitudes allocate more energy to rapid growth and higher metabolic rates, and invest less energy in reproduction (Lardies and Bozinovic, 2006). In contrast, some individuals from the same population present slow growth and lower metabolism, which allow mothers to invest more energy in offspring production. We suggest that the Mediterranean habitat of Santiago, which is characterized by high seasonal variation in food availability, temperature, and precipitation (Jaksic, 2001), produces this polymorphism in the Santiago population. This suggests that in populations from Santiago the covariation between reproductive output and metabolic costs has a genetic basis, since differences among populations were maintained in the F<sub>1</sub> generation. This would also allow for an assessment of development time, which is very important for adaptation to season length, as opposed to temperature in an invasive species (Blanckenhorn, 1997). Interestingly, the trade-offs and positive correlations observed for Santiago were absent in Antofagasta and La Serena (Table 3). This can be

explained by the plasticity of trait correlations, where the correlation between two traits can be altered by the environment (Schlichting and Pigliucci, 1998).

Differences in physiological and life-history traits in offspring can also be due to maternal effects (Mousseau and Fox, 1998; see also McAdam *et al.*, 2002; David *et al.*, 2003; Lardies *et al.*, 2004a; Bacigalupe *et al.*, 2007). Terrestrial isopods undergo direct development (Hoese and Janssen, 1989; Surbida and Wright, 2001). Thus, there is a great opportunity for environmental maternal effects to be present. Our experimental design cannot discard the presence of maternal effects; however, the incidence of maternal effects has been reported to diminish rapidly with offspring age, and has little or no influence on adult offspring phenotype (e.g. Cheverud and Moore, 1994; Hunt and Simmons, 2000). Furthermore, mean values of the different traits were coincident with the patterns of natural variation of these traits (see Castañeda *et al.*, 2004, 2005; Lardies and Bozinovic, 2006).

### Genetic variation in phenotypic plasticity

The significant population (genotype)  $\times$  environment interaction in all studied life-history and metabolic traits indicates that there is genetic variation for phenotypic plasticity in response to cold or warm environments (Figs. 1, 2, and 3). This observation was likely due to adaptive phenotypic plasticity, with females experiencing high reproductive output with a smaller number of offspring of larger size. Environmental and genetic sources tended to affect offspring size to a similar extent, emphasizing the importance of considering both types of variation (Sinervo and Svensson, 1998; Niewiarowski, 2001). Although temperature-mediated plasticity in body size is a widespread phenomenon, an adjustment in offspring size and reproductive output to the prevailing temperature conditions could be of particular importance for temperate populations of *P. laevis* inhabiting the most seasonal environment (i.e. Santiago), since these populations show the highest degree of plasticity.

We agree with the suggestion that the degree of phenotypic plasticity may vary among populations as well as the presence of geographical differences in plasticity (Bronikowski and Arnold, 1999; Seigel and Ford, 2001; Hassall *et al.*, 2005). Metabolic rate, reproductive output, and offspring size and number in terrestrial isopods from Santiago were significantly plastic in response to both environments, whereas the same traits were less plastic in the low-latitude populations (Antofagasta and La Serena). Thus, geographical variation in the degree of plasticity for these traits has been demonstrated within a single species. It is important to note that we cannot determine whether phenotypic plasticity is absent in Antofagasta and La Serena, since it is possible that experimental environments of lower or higher temperature could produce significant plasticity.

Considerable time and effort have been invested in examining geographical differences in life-history traits in arthropods (Fox and Czesak, 2000). However, it has been argued elsewhere that much of the observed geographical differences in life-history traits among populations may be the result of phenotypic plasticity, and not of natural selection to the local environment (Stearns, 1992). Our data show that the observed differences in life-history and metabolic traits between localities are mainly a function of genetic differences. However, the sites differ in their response to the environment, as shown by the significant interaction term in all of the studied life-history traits (see Figs. 1 and 2). Plasticity of reproductive investment arising from food or temperature effects can be considered a physiological constraint, which is uninteresting from an adaptive point of view (Via *et al.*, 1995). However, plasticity in allocation (reproductive output), number and/or size of offspring could be adaptive in the sense that it may lead to increased fitness under varying environments. Our data imply that

adaptive explanations for geographical differences in life-history traits *per se* for this species may be misdirected. Alternatively, more attention should be given to understanding geographical differences in variability rather than differences in mean values.

Since gene flow in terrestrial isopods is known to be low or non-existent (Gentile and Sbodorni, 1998), this genetic isolation of populations combined with different selection pressures among habitats has provided the opportunity for natural selection to shape population-specific differences. As a consequence, substantial variability in many traits exists among isopod populations (Hassall *et al.*, 2005; Pörtner *et al.*, 2006), which – through the use of invasive species as model organisms – may reflect replicated experiments of evolution (Huey *et al.*, 2005). Additionally, results from F<sub>2</sub> generations are needed to confirm that this variability is due to genetic differences among populations.

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