

Phenotypic covariance matrix in life-history traits along a latitudinal gradient: a study case in a geographically widespread crab on the coast of Chile

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ABSTRACT: Geographically widely spread species can cope with environmental differences among habitats by genetic differentiation and/or phenotypic flexibility. In marine crustaceans, intraspecific variations in life-history traits are pervasive along latitudinal clines. Replicated latitudinal clines are of evolutionary interest because they provide evidence of the occurrence of natural selection. If the means of traits along the latitudinal gradient are expected to be the result of natural selection, there is no reason why variances and covariances will not also be subject to selection, since selection is essentially a multivariate phenomenon. We studied life-history changes in means, variances, and covariances (i.e. **P** matrix) in 6 populations of the endemic crab *Cyclograpsus cinereus* (Decapoda: Grapsidae) along a latitudinal gradient over 19° on the Chilean coast. Trait means differed among localities for all traits analyzed (i.e. female size, number and size of eggs, and reproductive output), and the variation displayed a clinal pattern. In general, the main result that emerged from planned comparisons of **P** matrices is that, when detected, differences between localities mainly reflect differences in the magnitude of phenotypic variation (i.e. eigenvalues), rather than in the relationships between traits (i.e. eigenvectors). Sea-surface temperature was only correlated with the covariance between egg numbers and reproductive output. Matrices comparisons for Flury and jackknife methods were highly linked, with limits of biogeographic provinces described for the coast of Chile. Our study strongly highlights the importance of estimating the **P** matrix, not only mean values, in order to understand the evolution of life-history traits along a latitudinal gradient. Furthermore, the study of the variation in the **P** matrix might provide important insights into those evolutionary forces acting on it.

KEY WORDS: Intertidal · Reproduction · Crustacea · Plasticity · Clinal variation

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INTRODUCTION

Species distributed across broad geographic ranges often display extensive variation in life-history phenotypes; the explanations for such differences have been a matter of extensive theoretical and empirical work (see Niewiarowski 2001, Bernardo & Reagan-Wallin 2002, Roff 2002). Components of life-history are generally viewed as a collection of coadapted traits, which have been molded by natural selection (Stearns 1992; see also Fox & Czesak 2000, Lardies & Castilla 2001,

Ricklefs & Wikelski 2002). Environmental gradients are common place in nature and are broadly recognized to exert a major effect on patterns of variation within species (Mizera & Meszéna 2003). In this sense, the variability in life-history traits among populations along a latitudinal gradient is thought to be genetically based and therefore to reflect differences among environments and/or phenotypic plasticity, which is largely environmentally determined (Via & Lande 1985, Loeschcke et al. 2000; but see also Conover & Schultz 1995).

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However, geographic variation is in itself no proof of the action of natural selection: drift, gene flow, and phenotypic plasticity may also be responsible for clinal variation. Statistically, clinal variation has been evaluated by correlating trait means with different environmental (e.g. temperature) or geographical (e.g. latitude) variables along the gradient. Although this approach gives insight into the mean response of life-history traits along a gradient, selection and evolution are essentially multivariate phenomena, and, thus, aspects of the complex relationship among variables cannot be addressed in a unique analysis. The evolution of a multivariate phenotype can be modeled using the breeder's equation $\Delta\bar{z} = \mathbf{G}\mathbf{P}^{-1}\mathbf{S}$ where $\Delta\bar{z}$ is the vector of change in mean trait values, \mathbf{G} is the matrix of additive genetic variances and covariances, \mathbf{P} is the phenotypic variance–covariance matrix, and \mathbf{S} is the vector of selection differentials (Lande & Arnold 1983). It is clear from the above equation that any change in means ($\Delta\bar{z}$), e.g. associated with a cline, will always be a function of the phenotypic relationships that exist among the particular traits (i.e. \mathbf{P} matrix). Furthermore, an unresolved issue in evolutionary biology is whether the \mathbf{P} matrix reflects historical and/or developmental constraints, or whether it is determined by past selective pressure on the same phenotypic traits (Cheverud 1984, Arnold et al. 2001). If means along a cline are expected to be the result of natural selection, there is no reason why phenotypic variances and covariances between traits will not also be subject to selection or other evolutionary forces (Roff & Mousseau 2005, Eroukhanoff & Svensson 2008). Understanding variation in the \mathbf{P} matrix might provide important insights on the forces that are acting upon it.

In the 1950s, Thorson (1950) established that marine invertebrates have a shorter larval phase towards the Poles than those at lower latitudes. In parallel, other ecological and evolutionary patterns have been described, for example Bergmann's rule (Atkinson & Sibly 1997), which predicts an increase in body size in areas with colder climates (i.e. higher latitudes/altitudes). These differences were considered to be a result of many environmental factors that varied with latitude, e.g. temperature, primary productivity, and habitat (Lardies & Castilla 2001, Lardies et al. 2008 and references therein). However, the reasons behind the latitudinal cline in the reproductive biology of marine invertebrates are still under discussion (Gallardo & Penchaszadeh 2001). In this context, 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 & Czesak 2000, Lardies & Castilla 2001,

Ricklefs & Wikelski 2002). However, the most likely candidate as a selective agent is temperature (Robinson & Partridge 2001, Fischer et al. 2004). Due to the presence of the Humboldt Current System (HCS), the physical characteristics of Chilean coastal water masses are relatively similar along the coast (Viviani 1979, Thiel et al. 2007). This favors a wide distribution of many marine invertebrates (Brattström & Johanssen 1983), including decapod crustaceans (Holthuis 1952, Wehrtmann & Carvacho 1997, Thiel et al. 2007). Here, we studied life-history changes in means and in the phenotypic covariance matrix in 6 populations of the endemic crab *Cyclograpsus cinereus* (Decapoda: Grapsidae) along a latitudinal gradient along 19° of the Chilean coast. *C. cinereus* inhabits the high rocky shore and spends most of the tidal cycle in the air; during neap tides and low wave action, many individuals may rest on dry sand under boulders (Bahamonde & Lopez 1969). Our objectives were 2-fold. (1) To evaluate whether phenotypic means and \mathbf{P} vary along the latitudinal gradient in a clinal pattern. (2) To evaluate if variation in means and in the \mathbf{P} matrix correlates with sea-surface temperature and/or sea productivity, which may suggest that natural selection is responsible for such variation.

MATERIALS AND METHODS

Crabs and study site. Specimens of *Cyclograpsus cinereus* were collected in the intertidal zone from the following localities along the coast of Chile: Antofagasta, Coquimbo, El Tabo, Lenga, Playa Rosada (Valdivia), and Puerto Montt (for details on locations and environmental variables see Fig. 1 and Table 1). The latitudinal range that was sampled spanned approximately 2400 km; individuals were collected by hand at low tide during the autumn and spring of 2007.

Analysis of *Cyclograpsus cinereus* females and eggs. Females of *C. cinereus* covering the whole size range for the species were collected from the rocky intertidal. Crabs were placed in individual, labeled bottles containing a fixative solution of 10% formaldehyde (diluted with seawater). Carapace length (CL) was measured for each crab using a stereomicroscope with a calibrated eyepiece. Dry body mass (m_b) was also determined using an analytical balance with a precision of ± 0.01 mg. For this procedure, individuals were rinsed twice with distilled water and dried for 72 h at 60°C. Eggs were previously removed from each female, and their number (EN) was determined. Egg lengths and widths were measured for 20 eggs female⁻¹ and were used to calculate egg volume (EV) using the formula for the volume of an ellipsoid (see Lardies & Wehrtmann 1997). All eggs were counted,



Fig. 1. Location of 6 study sites for individuals of *Cyclograpsus cinereus* collected in 2007 along the coast of Chile

staged, and measured under a stereomicroscope using a calibrated ocular micrometer. The developmental stages of the eggs were also determined following the criteria proposed by Lardies & Wehrtmann (1997)—Stage I: eggs recently produced, uniform yolk, no visible eye pigments; Stage II: eye pigments barely visible; and Stage III: eyes clearly visible and fully developed, abdomen free. Egg volume comparisons among populations were only considered for eggs in Stage I. Considering that crustaceans frequently lose eggs during their development (see Kuris 1991), the individual egg number was estimated only in females with eggs in Stage I (recently produced). Dry egg mass was finally determined following the methodology for m_b described above. With this information, the reproductive output (RO) or biomass invested on reproduction by each female was estimated according to Clarke et al. (1991).

Analysis of phenotypic means. Egg volume and egg numbers were \log_{10} -transformed to meet normality assumptions. We compared phenotypic means among localities with a 1-way ANCOVA, using body length as a covariate. When differences were detected, means were compared according to the latitudinal gradient of localities with the following planned comparisons: (1) Antofagasta versus Coquimbo, (2) Coquimbo versus El Tabo, (3) El Tabo versus Lenga, (4) Lenga versus Playa Rosada, and (5) Playa Rosada versus Puerto

Montt. We applied a Bonferroni correction as we tested 3 response variables on the same dataset. The new α was set at 0.017. Data are presented as means (\pm SD).

Matrix comparisons: Flury hierarchy and jackknife-MANOVA. Because there is no clear consensus on the best methodology to compare matrices (Stephan et al. 2002), we used both Flury and jackknife-MANOVA methods to address different aspects of matrix variation. The Flury approach addresses a series of hierarchical hypotheses regarding matrix similarity, testing whether matrices are either equal, proportional (matrices differ in the magnitude of their variances by a proportional constant, but they share all principal components, i.e. all axes have the same orientation), share common principal components (all or some axes have the same orientation), or are unrelated (Phillips & Arnold 1999, Roff 2000). We used the jump-up procedure to test each of the degrees of matrix similarity against the model of unrelated structure (Phillips & Arnold 1999). To avoid the

assumption of multivariate normality we carried out hypothesis testing with randomization. In this analysis, 4999 data sets were created, with individuals randomized across localities. This analysis was performed with the program CPCrand (Phillips & Arnold 1999). The jackknife-MANOVA method first estimates the phenotypic covariance matrix (i.e. \mathbf{P}) for each locality, then one individual in turn is deleted and the pseudovalues for each component of \mathbf{P} are calculated according to the jackknife procedure (Roff 2006). Finally, all

Table 1. Latitude, sea-surface temperature, sea productivity (chl *a*), and number of individuals sampled (*n*) for the 6 populations of *Cyclograpsus cinereus* studied along the Chilean coast. Data for sea-surface temperature are from SHOA (2006); data for chl *a* are from Thiel et al. (2007). Mean values are indicated

Locality	Latitude (°S)	Sea-surface temperature (°C)	Chl <i>a</i> (mg C m ⁻³)	<i>n</i>
Antofagasta	23° 31'	18.61	0.76	35
Coquimbo	29° 56'	16.01	1.17	46
El Tabo	33° 24'	15.26	1.31	47
Lenga	36° 31'	14.24	3.02	25
Playa Rosada (Valdivia)	39° 46'	13.55	2.38	44
Puerto Montt	41° 29'	12.99	1.26	24

pseudovalue are used as dependent variables in a MANOVA, whereby locality is used as the independent variable. When differences among all localities were detected, **P** matrices were also compared according to their latitudinal gradient with the same *a priori* planned comparisons as given in 'Analysis of phenotypic means'. For egg numbers and volume, we used residuals from a linear regression against body length.

Correlations between means and elements of **P with temperature and sea productivity.** A Pearson correlation was used to evaluate the association between means, variances, and covariances with average sea-surface temperature integrated over the last 40 yr and the mean amount of chlorophyll *a* (a proxy for sea productivity) (see Table 1). We used a single population estimate for each mean, variance, and covariance. Elements of **P** (variances and covariances) were the jackknifed estimates. Note that we decided not to evaluate the effect of latitude in addition to the effect of temperature, since both traits were—as expected—highly correlated ($r_p = 15$ to 0.99, $p < 0.001$).

RESULTS

Analysis of phenotypic means

Descriptive statistics of studied traits are presented in Figs. 2 & 3. Means differed among localities for all traits (see Table 2). Overall, the results of the planned comparisons (Table 2) indicate a clear pattern: egg numbers and RO were statistically different only in the Playa Rosada versus Puerto Montt comparison, where there is a major biogeographical change in the coast (i.e. the limit between the intermediate zone and the Magellan province) (Fig. 1). On the other hand, egg volume and body size showed a constant increase along the coast of Chile, from low to high latitudes, although for egg volume some of the comparisons were only marginally significant (Fig. 2, Table 2).

Matrix comparisons I: Flury hierarchy

Phenotypic matrices for each locality are presented in Table 3. The Flury analysis indicates that **P** matrices differ among all localities ($p < 0.05$). In general, the main result that emerged from the pairwise planned comparisons of **P** matrices is that, when detected, differences between localities mainly reflect differences in the magnitude of phenotypic variation (i.e. eigenvalues), not in the relationships between traits (i.e. eigenvectors). Specifically, **P** matrices between: (1) Antofagasta and Coquimbo were proportional ($p = 0.169$), (2) Coquimbo and El Tabo were proportional ($p = 0.463$),

(3) El Tabo and Lenga were proportional ($p = 0.845$), (4) Lenga and Playa Rosada were equal ($p = 0.513$), and (5) Playa Rosada and Puerto Montt were proportional ($p = 0.063$).

Matrix comparisons II: Jackknife-MANOVA

The results of the MANOVA method indicated that **P** matrices differed among all localities (Wilks $\lambda = 0.698$, approximate $F_{30,842} = 2.636$, $p < 0.05$). Overall, the pattern detected with MANOVA in the pairwise planned comparisons of **P** matrices was very similar to the one

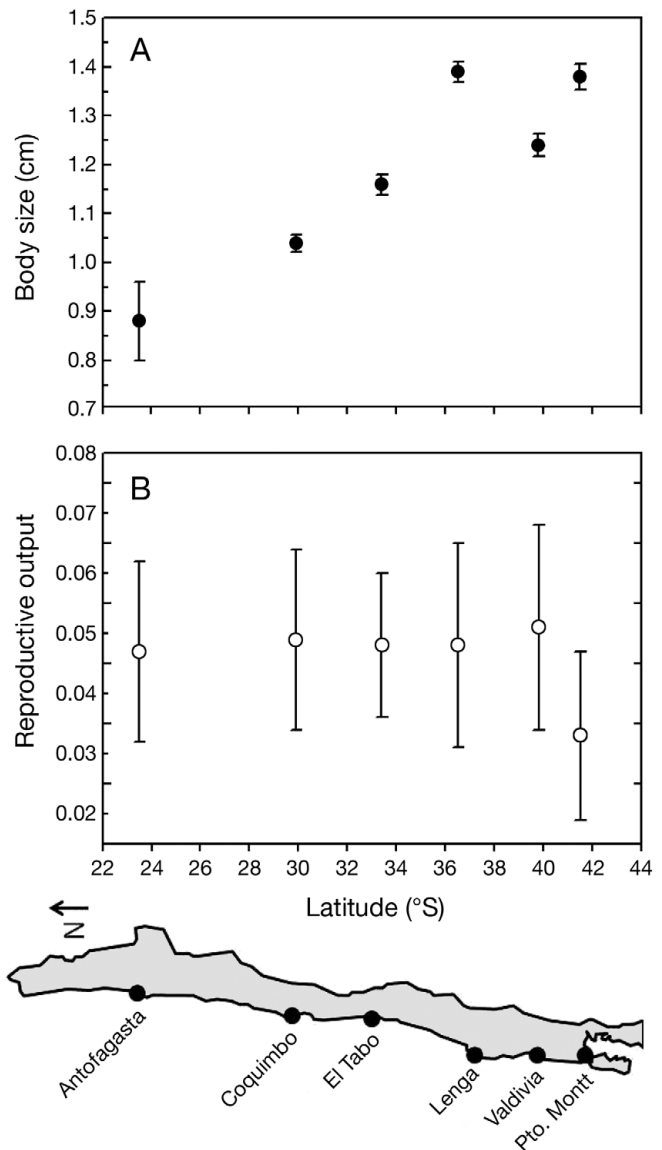


Fig. 2. *Cyclograpsus cinereus*. (A) Carapace length and (B) reproductive output of ovigerous females from different latitudes along the Chilean coast. Mean (\pm SD) values are indicated

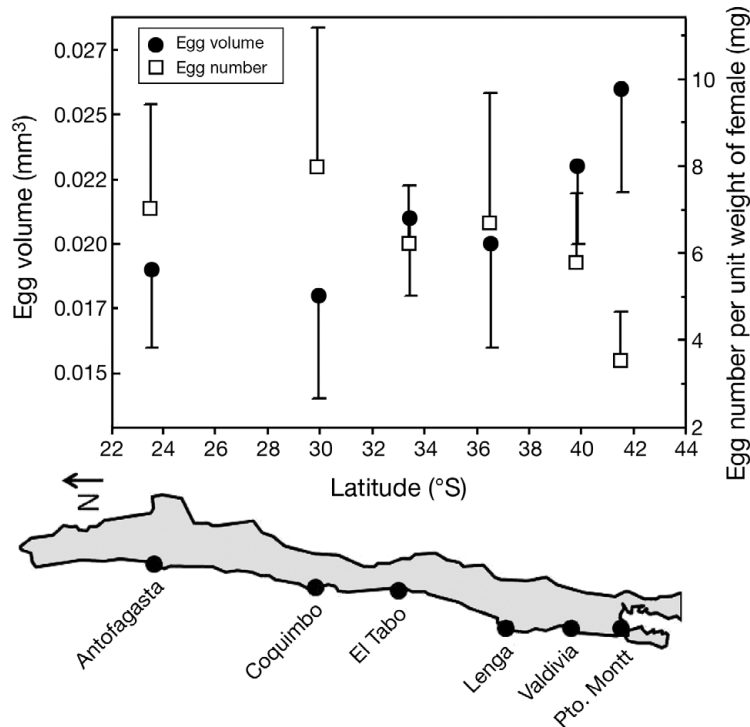


Fig. 3. *Cyclograpsus cinereus*. Mean egg volume (●) of freshly extruded eggs (mean – SD), and egg number (□) per unit weight of females (mean + SD; based on mg dry weight), for populations collected at different latitudes along a latitudinal cline on the Chilean coast

obtained by the Flury hierarchy. Specifically, **P** matrices differed only between the northern localities and only in the variance of egg numbers: (1) Antofagasta versus Coquimbo (Wilks $\lambda = 0.838$, approximate $F_{6,74} = 2.390$, $p = 0.036$), and (2) Coquimbo versus El Tabo (Wilks $\lambda = 0.790$, approximate $F_{6,86} = 2.511$, $p = 0.002$). Matrices did not differ between: (1) Tabo and Lenga (Wilks $\lambda = 0.854$, approximate $F_{6,65} = 1.845$, $p = 0.104$), (2) Lenga and Playa Rosada (Wilks $\lambda = 0.901$, approximate $F_{6,62} = 1.131$, $p = 0.356$), or (3) Playa Rosada and Puerto Montt (Wilks $\lambda = 0.845$, approximate $F_{6,61} = 1.858$, $p = 0.103$).

Correlations between means and elements of **P** with temperature and sea productivity

Regarding mean values, sea temperature was negatively associated with body length ($r_p = -0.93$, $p = 0.008$) and sea productivity was positively correlated with egg numbers ($r_p = 0.93$, $p = 0.007$). Regarding the elements of **P**, temperature was positively correlated with the covariance between egg numbers and RO ($r_p = 0.84$, $p = 0.036$) and negatively correlated with the covariance between egg volume and egg numbers ($r_p = -0.87$, $p = 0.026$).

DISCUSSION

Phenotypic means

A clinal pattern of variation in body size in *Cyclograpsus cinereus* has been reported for several ectotherms (Jones & Simons 1983, France 1992, Lardies & Bozinovic 2008, and references therein). Diverse ecological variables are likely to be selective agents, with temperature being a key one (Fischer & Fiedler 2002, but see also Blanckenhorn & Demont 2004). Here we found that body mass of *C. cinereus* increased with latitude (Table 2, Fig. 2), a pattern that is in agreement with information for other crustacean species (Lardies & Castilla 2001, Lardies & Wehrtmann 2001, Lardies et al. 2008). Our results support Bergmann's rule, which predicts an increase in body size at higher latitudes or altitudes (Laptikhovskiy 2006) and suggests temperature as a potential selective factor with regards to body size (see Fig. 2). Furthermore, *C. cinereus* females from lower latitudes are smaller when they start producing eggs than females from higher latitudes, which is in agreement with previous observations in several species of crustaceans (Jones & Simons 1983, Lardies & Castilla 2001, Lardies et al. 2008). Temperature has also been recognized as a factor stimulating growth and early ovarian development (Nelson et al. 1988a,b). Nevertheless, another factor that may explain the observed variation in size in crustaceans is the nutritional state of females (Harrison 1990).

The number of offspring in equally sized ovigerous females increased significantly from high to low latitudes ($r_p = -0.77$, $p < 0.001$). On the other hand, the egg volume increased linearly towards high latitudes ($r_p = 0.82$, $p < 0.05$) (see Fig. 3). This trade-off is known to occur in many invertebrates (Clarke et al. 1991, France 1992, Fox & Czesak 2000, Wilhelm & Schindler 2000). 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 RO (i.e. egg mass/female mass) in *Cyclograpsus cinereus* increases towards high latitudes, which consequently increases offspring size and total number. In addition, egg size is considered to be a good estimator of egg energy content in crustaceans (Clarke 1993). Accordingly, *C. cinereus* eggs 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 colder regions (Bauer 1992, Blanckenhorn

Table 2. *Cyclograpsus cinereus*. Planned comparisons of the traits of crabs analyzed among the localities studied

Trait Comparison	SS	df	MS	F	p
Egg volume (mm³)					
Locality	0.337	5	0.067	9.670	<0.001
Antofagasta–Coquimbo	0.051	1	0.051	7.359	0.007
Coquimbo–El Tabo	0.086	1	0.086	12.288	<0.001
El Tabo–Lenga	0.023	1	0.023	3.271	0.072
Lenga–Playa Rosada	0.071	1	0.071	10.227	0.002
Playa Rosada–Puerto Montt	0.026	1	0.026	3.791	0.053
Residual	1.490	214	0.007		
Reproductive output					
Locality	0.0052	5	0.0010	4.618	<0.001
Antofagasta–Coquimbo	0.00005	1	0.00005	0.197	0.657
Coquimbo–El Tabo	0.00001	1	0.00001	0.031	0.861
El Tabo–Lenga	0.000002	1	0.000002	0.008	0.928
Lenga–Playa Rosada	0.0001	1	0.0001	0.628	0.429
Playa Rosada–Puerto Montt	0.0047	1	0.0047	20.547	<0.001
Residual	0.049	215	0.0002		
Egg number (per unit weight of female mg)					
Locality	1.067	5	0.214	8.10	<0.001
Antofagasta–Coquimbo	0.019	1	0.019	0.723	0.360
Coquimbo–El Tabo	0.002	1	0.002	0.079	0.779
El Tabo–Lenga	0.002	1	0.002	0.067	0.796
Lenga–Playa Rosada	0.021	1	0.021	0.807	0.370
Playa Rosada–Puerto Montt	0.584	1	0.584	22.169	<0.001
Residual	5.637	214	0.026		
Body size (cm)					
Locality	0.889	5	0.178	49.92	<0.001
Antofagasta–Coquimbo	0.095	1	0.095	26.55	<0.001
Coquimbo–El Tabo	0.052	1	0.052	14.54	<0.001
El Tabo–Lenga	0.107	1	0.107	30.16	<0.001
Lenga–Playa Rosada	0.039	1	0.039	10.99	<0.01
Playa Rosada–Puerto Montt	0.021	1	0.021	5.88	0.016
Residual	0.766	215	0.004		

1997). Since the size of an offspring (egg) and the number of eggs produced are usually opposing attributes, selection for one attribute will forfeit the other (Mousseau & Fox 1998).

Most available information suggests that the RO of polar species is lower than that of species inhabiting temperate zones (Clarke 1987, Lardies & Wehrmann 2001). This contradicts our findings showing that the RO of *Cyclograpsus cinereus* increased at higher latitudes, as well as findings by others reporting similar patterns for other marine crustaceans (Lardies & Castilla 2001, Lardies et al. 2008). In the present study, low-latitude females invest about 4.7% of their body mass in egg production compared with 5.1% of females at higher latitudes. Our results indicate that *C. cinereus* females collected from high latitudes invest more biomass in egg production than females from low latitudes. This suggests that individuals at high temperatures concentrate more energy on rapid growth and high metabolism, and invest less energy in reproduction (Díaz 1980, Lardies & Bozinovic 2006). Inversely, the slow growth and low metabolism of

species in zones with low temperatures (Brey & Clarke 1993) allow more energy investment in egg production, since the eggs contain larger internal reserves (Woodward & White 1981, Lardies & Bozinovic 2008). Strong support of the previous idea was found in differences in the metabolism of populations of *C. cinereus* along the coast of Chile; high metabolism was observed at low latitudes compared with low metabolism of populations at high latitudes (Lardies et al. in press). Despite the fact that the quantity of eggs produced by equally sized crab females was greater in the low-latitude populations, RO was larger in the intermediate- and high-latitude populations. Unfortunately, the number of egg batches produced per year for each population is unknown, and, therefore, we cannot estimate the annual RO for each of the study populations, which could change the direction of our results.

Latitudinal clines in ectotherms' traits are largely caused by the environment. Seasonal variation in water temperature, a pattern that is strongly tied to latitude (see Table 1), has always been considered an important proximate factor or environmental stimulus triggering and maintaining gametogenesis and other reproductive characteristics of marine invertebrates. In accordance with several authors (Thorson 1950, Pearse et al. 1991, Bauer 1992, Brante et al. 2004), we believe that the important selective pressure acting on reproductive traits (i.e. size of reproduction, egg number, and RO) in marine invertebrates with planktotrophic larvae might be the temporal variation of larval food supply; that is, the seasonal pattern of primary and secondary productivity. Thus, the temporal availability of the larval food supply has been reported to be the ultimate factor for the observed variation in several aspects of the reproductive biology of benthic invertebrates (Bauer 1992, Olive 1992, Thiel et al. 2007). Although the temporal variation in sea-surface temperature and sea productivity in the studied populations is unknown, the patterns of seasonal temperature and productivity are stronger on the southern coast of Chile than on the northern coast (Thiel et al. 2007). Hence, temperature (proximate factor) may well be correlated with hydrographic conditions, leading to

Table 3. Phenotypic matrices for each locality. Variances (diagonal) and covariances (above diagonal) were the jackknifed estimates obtained with transformed and size-corrected data. Phenotypic correlations (SE) are depicted below the diagonal of variances. RO: reproductive output

	RO	Egg number	Egg volume
Antofagasta			
RO	0.0002	0.0021	-0.0005
Egg number	0.83 (0.07)	0.028	-0.0046
Egg volume	-0.43 (0.13)	-0.36 (0.14)	0.0061
Coquimbo			
RO	0.0002	0.0012	-0.0006
Egg number	0.50 (0.17)	0.028	-0.005
Egg volume	-0.35 (0.16)	-0.31 (0.17)	0.013
El Tabo			
RO	0.00014	0.00048	0.00001
Egg number	0.40 (0.16)	0.0104559424	-0.0020
Egg volume	0.005 (0.13)	-0.29 (0.14)	0.0048
Lenga			
RO	0.00029	0.0012	-0.0002
Egg number	0.48 (0.28)	0.0278	-0.001
Egg volume	-0.12 (0.26)	-0.04 (0.29)	0.0077
Playa Rosada			
RO	0.00028	0.00061	-0.00038
Egg number	0.23 (0.19)	0.0219	0.0011
Egg volume	-0.35 (0.11)	0.13 (0.14)	0.0041
Puerto Montt			
RO	0.00020	-0.0005	0.00008
Egg number	-0.14 (0.26)	0.0603	0.0018
Egg volume	0.06 (0.26)	0.10 (0.19)	0.0053

the variations in larval food supply that account for the clinal variation in the reproductive traits of *Cyclograpsus cinereus*.

Phenotypic variation and the evolution of the P matrix

Phenotypic variation in trait means is a common observation among geographically separated populations. When differences among populations are maintained under common garden conditions, it is usually suggested they have a genetic basis and, thus, are a consequence of natural selection. Although a response to selection depends on the genotype, selection acts on the overall phenotype, i.e. on the means and \mathbf{P} (e.g. Roff & Mousseau 2005). Thus, there is little reason to suppose that the elements of \mathbf{P} will not be subject to selection if mean trait values are expected to evolve. According to the multivariate version of the breeders' equation, the evolution of \mathbf{P} can result as an indirect consequence of the evolution of the mean phenotype, or as a consequence of the non-linear selection acting directly on it (Bacigalupe et al. 2008). Although we did not carry out a common garden design (i.e. ovigerous females were sampled from the field), the clear pattern

between mean trait values and sea-surface temperature strongly suggests that natural selection influences it.

Regarding \mathbf{P} , results show that the relationships between traits are maintained among localities. Pairwise comparisons suggest that differences between localities are mainly the consequence of the magnitude of phenotypic variation, as reflected in their shared principal components structure (i.e. orientation of axes) in both methods of analysis (MANOVA and Flury hierarchy). Furthermore, out of 6 components (i.e. 3 variances and 3 covariances, see Table 3), only 2 showed a relationship with environmental variables. First, sea-surface temperature was positively associated with the covariance between egg number and RO. That is, from south to north, the covariance became much stronger, which suggests that females that invest less in reproduction produce more eggs per unit of mass in the northern populations in comparison to the southern ones. Probably, this covariation between RO and egg number indicates a gradual interlink between traits that is stronger in northern populations. Second, sea-surface temperature was negatively associated with the covariance between egg volume and egg number. As mentioned earlier, the trade-off between egg number and size is a widespread pattern in marine invertebrates, and a negative correlation of this covariance with temperature indicates that a decline in temperature increases egg volume and, consequently, reduces egg number, because the area in a female's cephalothorax for carrying eggs is physically limited (see Hines 1992).

If selection were responsible for the latitudinal trend in means, we would have also expected an associated pattern in variances and covariances (Brodie et al. 1995). Interestingly, comparisons of matrices according to both methods are highly linked with the limits of the biogeographic province described for the coast of Chile (see Camus 2001, Astorga et al. 2003, Thiel et al. 2007). Thus, provinces share biotas, which are defined mainly by hydrographic characteristics (i.e. Humboldt Current System) that occurred after glacial-tectonic processes during the Tertiary period (see Camus 2001). On the one hand, comparison of \mathbf{P} between Antofagasta and Coquimbo (a distance of 887 km) reveals that the matrices are unrelated and different for Flury and MANOVA methods. Moreover, the limit between the Peruvian province and the intermediate zone is located in Coquimbo (Fernández et al. 2000, Camus 2001). On the other hand, pairwise comparisons of \mathbf{P} matrices between Coquimbo and Playa Rosada show a great conservation of structure, in agreement with the existence of an intermediate zone on the coast of Chile (Camus 2001). Finally, the 2 southern localities (Playa Rosada and Puerto Montt), share all eigenvectors (i.e. orientation of axes), although they differ in the magni-

tude of some components (see 'Results') despite the short distance between them (210 km). Coincidentally, in Puerto Montt, the limit between the intermediate zone and Magellan province is found (see Camus 2001, Thiel et al. 2007). Furthermore, another likely explanation is that southern sites correspond to the geographical and ecological edge of the species' distribution (see Bahamonde & Lopez 1969), where, in general, the environmental conditions are marginal, resulting in the observed decrease of the studied traits. The difference in water temperature and sea productivity experienced by southern populations of *Cyclograpsus cinereus* (Playa Rosada and Puerto Montt; see Table 1) is small but the **P** matrices are significantly different (i.e. proportional), which strengthens the idea that Puerto Montt is close to the ecological limit of distribution for this species. Finally, *C. cinereus* is a semiterrestrial crab; therefore, the natural selection processes may not be the same as for subtidal crustaceans. For example, the effect of air temperature as a selective factor can be considerable in organisms that spend several hours out of water.

Overall, the consistent absence of covariation between elements of the **P** matrix and temperature, together with the conservation of structure it shows among the 6 populations, suggests that: (1) (directional) selection has not been important in its evolution; (2) genetic drift, gene flow, or environmental plasticity are responsible for the observed differences, and/or (3) non-linear modes of selection (e.g. stabilizing) are operating decoupled from the evolution of the mean phenotype. Only further work will disentangle all these possibilities. In general terms, our work strongly highlights the importance of estimating the **P** matrix, not only the mean values, in order to understand the evolution of life-history traits along a latitudinal gradient.

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LITERATURE CITED

- Arnold SJ, Pfrender ME, Jones AG (2001) The adaptive landscape as a conceptual bridge between microevolution and macroevolution. *Genetica* 112/113:9–32
- Astorga A, Fernández M, Boschi E, Lagos N (2003) Two oceans, two taxa and one mode of development: latitudinal diversity patterns of South American crabs and test for possible causal processes. *Ecol Lett* 6:420–427
- Atkinson D, Sibly RM (1997) Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends Ecol Evol* 12:235–239
- Bacigalupe LD, Crudgington HS, Slate J, Moore AJ, Snook RR (2008) Sexual selection and interacting phenotypes in experimental evolution: a study of *Drosophila pseudo-obscura* mating behavior. *Evolution* 62:1804–1812
- Bahamonde N, Lopez MT (1969) *Cyclograpsus cinereus* Dana, en biocenosis supramareales de Chile (Crustacea Decapoda, Brachyura, Grapsidae). *Bol Mus Nac Hist Nat (Santiago)* 29:165–204
- Bauer RT (1992) Testing generalizations about latitudinal variation in reproduction and recruitment patterns with sicyoniid and caridean shrimp species. *Invertebr Reprod Dev* 22:193–202
- Bernardo J, Reagan-Wallin NL (2002) Plethodontid salamanders do not conform to general rules for ectotherm life histories: insights from allocation models about why simple models do not make accurate predictions. *Oikos* 97:398–414
- Blanckenhorn WU (1997) Altitudinal life history variation in the dung flies *Scathophaga stercoraria* and *Sepsis cynipsea*. *Oecologia* 109:342–352
- Blanckenhorn WU, Demont M (2004) Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integr Comp Biol* 44:413–424
- Brante A, Cifuentes C, Pörtner HO, Arntz W, Fernández M (2004) Latitudinal comparisons of reproductive traits in five brachyuran species along the Chilean coast. *Rev Chil Hist Nat* 77:15–27
- Brattström H, Johanssen A (1983) Ecological and regional zoogeography of the marine benthic fauna of Chile. Report No. 49 of the Lund University Chile expedition 1948–49. *Sarsia* 68:289–339
- Brey T, Clarke A (1993) Population dynamics of marine benthic invertebrates in Antarctic and sub-Antarctic environments: Are there unique adaptations? *Antarct Sci* 5:253–266
- Brodie EDIII, Moore AJ, Janzen FJ (1995) Visualizing and quantifying natural selection. *Trends Ecol Evol* 10:313–318
- Camus PA (2001) Biogeografía marina de Chile continental. *Rev Chil Hist Nat* 74:587–617
- Cheverud J (1984) Quantitative genetics and developmental constraints on evolution by selection. *J Theor Biol* 110:155–171
- Clarke A (1987) Temperature, latitude and reproductive output. *Mar Biol* 38:89–99
- Clarke A (1993) Egg size and egg composition in polar shrimps (Caridea: Decapoda). *J Exp Mar Biol Ecol* 168:189–203
- Clarke A, Hopkins CCE, Nilssen EM (1991) Egg size and reproductive output in the deepwater prawn *Pandalus borealis* Kroyer, 1838. *Funct Ecol* 5:724–730
- Conover DO, Schultz ET (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends Ecol Evol* 10:248–252
- Díaz H (1980) The mole crab *Emerita talpoida* (Say): a case of changing life history pattern. *Ecol Monogr* 50:437–456
- Eroukhmanoff F, Svensson EI (2008) Phenotypic integration and conserved covariance structure in calopterygid damselflies. *J Evol Biol* 21:514–526
- Fernández M, Jaramillo E, Marquet PA, Moreno CA and others (2000) Diversity, ecology and biogeography of nearshore benthic ecosystems: an overview and needs for conservation. *Rev Chil Hist Nat* 73:629–662
- Fischer K, Fiedler K (2002) Reaction norms from age and size at maturity in response to temperature: a test of the compound interest hypothesis. *Evol Ecol* 16:333–349
- Fischer K, Bot ANM, Zwaan BJ, Brakefield PM (2004) Genetic and environmental sources of egg size in the butterfly *Bicyclus anynana*. *Heredity* 92:163–169
- Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. *Annu Rev Entomol* 45:341–369

- France RL (1992) Biogeographical variation in size-specific fecundity of the amphipod *Hyalella azteca*. *Crustaceana* 62:240–248
- Gallardo CS, Penchaszadeh P (2001) Hatching mode and latitude in marine gastropods; revisiting Thorson's paradigm in the Southern Hemisphere. *Mar Biol* 138:547–552
- Harrison KE (1990) The role of nutrition in maturation, reproduction, and embryonic development of decapod crustaceans: a review. *J Shellfish Res* 9:1–28
- Hines AH (1992) Constraint on reproductive output in brachyuran crabs: pinnotherids test the rule. *Am Zool* 32:503–511
- Holthuis LB (1952) Reports of the Lund University Chile Expedition 1948–49. 5. The Crustacea Decapoda Macrura of Chile. *Lund Univ Arsskr NF Avd 2*, 47:1–109
- Jones MB, Simons MJ (1983) Latitudinal variation in reproductive characteristics of a mud crab, *Helice crassa* (Grapsoidea). *Bull Mar Sci* 33:656–670
- Kuris AM (1991) A review of patterns and causes of crustacean brood mortality. In: Wenner A, Kuris A (eds) *Crustacean egg production*. *Crustac Issues* 7:117–141
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 36:1210–1226
- Laptikhovskiy V (2006) Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. *PSZNI: Mar Ecol* 27:7–14
- Lardies MA, Bozinovic F (2006) Geographic covariation between metabolic rate and life-history traits. *Evol Ecol Res* 8:455–470
- Lardies MA, Bozinovic F (2008) Genetic variation for plasticity in physiological and life-history traits among populations of an invasive species, the terrestrial isopod *Porcellio laevis*. *Evol Ecol Res* 10:1–16
- Lardies MA, Castilla JC (2001) Latitudinal variation in the reproductive biology of commensal crab *Pinnaxodes chilensis* (Decapoda: Pinnotheridae) along the Chilean coast. *Mar Biol* 139:1125–1133
- Lardies MA, Wehrtmann IS (1997) Egg production in *Betaeus emarginatus* (H. Milne Edwards, 1837) (Decapoda: Alpheidae): fecundity, reproductive output and chemical composition of eggs. *Ophelia* 49:165–174
- Lardies MA, Wehrtmann IS (2001) Latitudinal variation in the reproductive biology of *Betaeus truncatus* (Decapoda: Alpheidae) along the Chilean coast. *Ophelia* 55:55–67
- Lardies MA, Medina M, Correa J (2008) Breakage of intraspecific patterns in coastal zones associated with copper mine tailings in Chile: the snapping shrimp *Betaeus emarginatus* as model. *Mar Ecol Prog Ser* 358:203–210
- Lardies MA, Muñoz JL, Paschke KA, Bozinovic F (in press) Latitudinal variation in the aeral/aquatic ratio of oxygen consumption of a supratidal high rocky-shore crab. *Mar Ecol-Evol Persp*
- Loeschcke V, Bundgaard J, Barker JS (2000) Variation in body size and life-history traits in *Drosophila aldrichi* and *D. buzzatii* from a latitudinal cline in eastern Australia. *Heredity* 85:423–433
- Mizera F, Meszéna G (2003) Spatial niche packing, character displacement and adaptive selection along an environmental gradient. *Evol Ecol Res* 5:363–382
- Mousseau TA, Fox CW (1998) *Maternal effects as adaptations*. Oxford University Press, Oxford
- Nelson K, Hedgecock D, Borgeson W (1988a) Factors influencing egg extrusion in the American lobster (*Homarus americanus*). *Can J Fish Aquat Sci* 45:797–804
- Nelson K, Hedgecock D, Borgeson W (1988b) Effects of reproduction upon molting and growth in female American lobsters (*Homarus americanus*). *Can J Fish Aquat Sci* 45:805–821
- Niewiarowski PH (2001) Energy budgets, growth rates, and thermal constraints: toward an integrative approach to the study of life-history variation. *Am Nat* 157:421–433
- Olive PJW (1992) The adaptive significance of seasonal reproduction in marine invertebrates: the importance of distinguishing between models. *Invertebr Reprod Dev* 22:165–174
- Pearse JS, McClintock JB, Bosch I (1991) Reproduction of Antarctic benthic marine invertebrates: tempos, modes, and timing. *Am Zool* 31:65–80
- Phillips PC, Arnold SJ (1999) Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution* 53:1506–1515
- Ricklefs RE, Wikelski M (2002) The physiology/life history nexus. *Trends Ecol Evol* 17:462–468
- Robinson SJW, Partridge L (2001) Temperature and clinal variation in larval growth efficiency in *Drosophila melanogaster*. *J Evol Biol* 14:14–21
- Roff D (2000) The evolution of the G matrix: Selection or drift? *Heredity* 84:135–142
- Roff DA (2002) *The evolution of life histories*. Chapman & Hall, New York, NY
- Roff DA (2006) *Introduction to computer-intensive methods of data analysis in biology*. Cambridge University Press, Cambridge
- Roff DA, Mousseau TA (2005) The evolution of the phenotypic covariance matrix: evidence for selection and drift in *Melanoplus*. *J Evol Biol* 18:1104–1114
- SHOA (Servicio Hidrográfico y Oceanográfico de la Armada de Chile) (2006) *Atlas oceanográfico de Chile*, Vol 1 (18°21'S a 50°00'S). SHOA, Valparaíso
- Stearns SC (1992) *The evolution of life histories*. Oxford University Press, Oxford
- Steppan SJ, Phillips PC, Houle D (2002) Comparative quantitative genetics: evolution of the G matrix. *Trends Ecol Evol* 17:320–327
- Thiel M, Macaya E, Acuña E, Arntz W and others (2007) The Humboldt Current System of northern and central Chile: oceanographic processes, ecological interactions and socio-economic feedback. *Oceanogr Mar Biol Annu Rev* 45:195–344
- Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biol Rev Camb Philos Soc* 25:1–45
- Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39:505–523
- Viviani CA (1979) *Ecografía del litoral chileno*. *Stud Neotrop Fauna Environ* 14:65–123
- Wehrtmann IS, Carvacho A (1997) New records and distribution ranges of shrimps (Crustacea: Decapoda: Penaeoidea and Caridea) in Chilean waters. *Proc Biol Soc Wash* 110:49–57
- Wilhelm FM, Schindler DW (2000) Reproductive strategies of *Gammarus lacustris* (Crustacea: Amphipoda) along an elevational gradient. *Funct Ecol* 14:413–422
- Woodward IO, White RGW (1981) Effects of temperature and food on the fecundity and egg development rates of *Boeckella symmetrica* Sars (Copepoda: Calanoidea). *Aust J Mar Freshwater Res* 32:997–1002