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Genetic variation of the shell morphology in *Acanthina monodon* (Gastropoda) in habitats with different wave exposure conditions

Maribel R. Solas[1], Roger D. Sepúlveda[2], Antonio Brante[1,*]

[1]Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Casilla 297, Concepción, Chile
[2]Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

ABSTRACT: Shell morphology in marine gastropods exhibits high intraspecific variability. Plasticity appears to be the most common response; however, additive genetic variance would explain a significant amount of morphological variance promoting local adaptations. The marine gastropod *Acanthina monodon* is a direct developer with a low dispersal potential and high variability in shell morphology. Previous studies suggest that adult shell morphology could have an adaptive value that responds to the degree of wave exposure. Given the low dispersal potential of *A. monodon* and the high temporal predictability of wave exposure conditions at the local scale, it was expected that selection would favour local adaptations with an important component of heritability. To test this hypothesis we estimated shell morphology heritability of *A. monodon* using pre-hatching juveniles from wave-exposed and wave-sheltered sites along the Chilean coast and using a geometric morphometrics approach. In addition, we compared shell morphology and size of pre-hatching juveniles between exposed and sheltered sites. Heritability of shell morphology was highly variable, ranging between 0.2 and 0.6 among sites. Shell morphology of pre-hatching individuals did not differ between exposed and sheltered conditions. These results suggest that the diversity of shell morphology in adults of *A. monodon* could be explained by a complex interaction between plastic and heritable responses.

KEY WORDS: Additive genetic variance · Phenotypic plasticity · Geometric morphometrics · Snail · Shell shape · Body size

INTRODUCTION

Natural selection can produce adaptations to different habitats in 2 ways: first, by direct action on the additive genetic component, and second, by phenotypic plasticity (Via 1993, Via et al. 1995, Kingsolver et al. 2002). Theoretical models suggest that the proportional influence of each component (genetic and plasticity) on a trait will depend on genetic flow intensity and environmental uncertainty (Bertness & Gaines 1993, Mopper et al. 1995, Holt & Gomulkiewicz 1997). Thus, the evolution of the optimal genetic/plastic ratio will depend on how individuals perceive the habitat. In species with high dispersal potential, a high proportion of heritability will evolve in environments with low spatial and temporal variability; on the other hand, a high proportion of plasticity would be advantageous in habitats with high spatial or temporal variability. In contrast, in species with low dispersal, high levels of inheritance would be advantageous in local environments with low temporal variability. Thus, the study of inheritance and plasticity evolution must incorporate both the environmental characteristics and life history strategies of species in order to understand adaptation and spe-

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*Corresponding author. Email: abrante@ucsc.cl ciation processes at ecological and evolutionary scales (Zelditch et al. 2004).

Morphology is usually a feature related to fitness, and is therefore restricted to strong selective pressures (Johannesson et al. 1993). Shell morphology of marine gastropods has been one of the most frequently studied morphological traits, due to its importance for taxonomic and evolutionary studies. At the intraspecific-level, shell morphology exhibits high polymorphism, which is explained by both environmental and biological factors. For example, the intertidal species *Littorina saxatilis* and *Nucella lapillus* exhibit a high degree of variability in shell morphology, resulting in several morphs or ecotypes apparently related to local selective pressures induced by environmental conditions such as predation and wave strength (Johannesson et al. 1993). In *N. lapillus* the shell is more globular at sites exposed to wave action and more elongated at sheltered sites, resulting in 2 ecotypes (Kitching et al. 1966, Crothers 1975, Rolán et al. 2004). Individuals from exposed sites are characterized by a more rounded aperture with a larger foot which facilitates a stronger attachment to the substrata (Kirby et al. 1994). Both species are direct developers, which means they lack an intermediate larval stage, and display a restricted crawling range. Moreover, genetic analyses indicate a low dispersal potential (Pascoal et al. 2012). The characteristics of the selective forces, such as wave strength, which usually exhibits high local predictability over time (a site may be classified as exposed or sheltered depending on the strength of temporal wave patterns), and their restricted dispersal suggest that the shell morphology of these species should have a high genetically additive base, with significant heritability values. In fact, for *L. saxatilis*

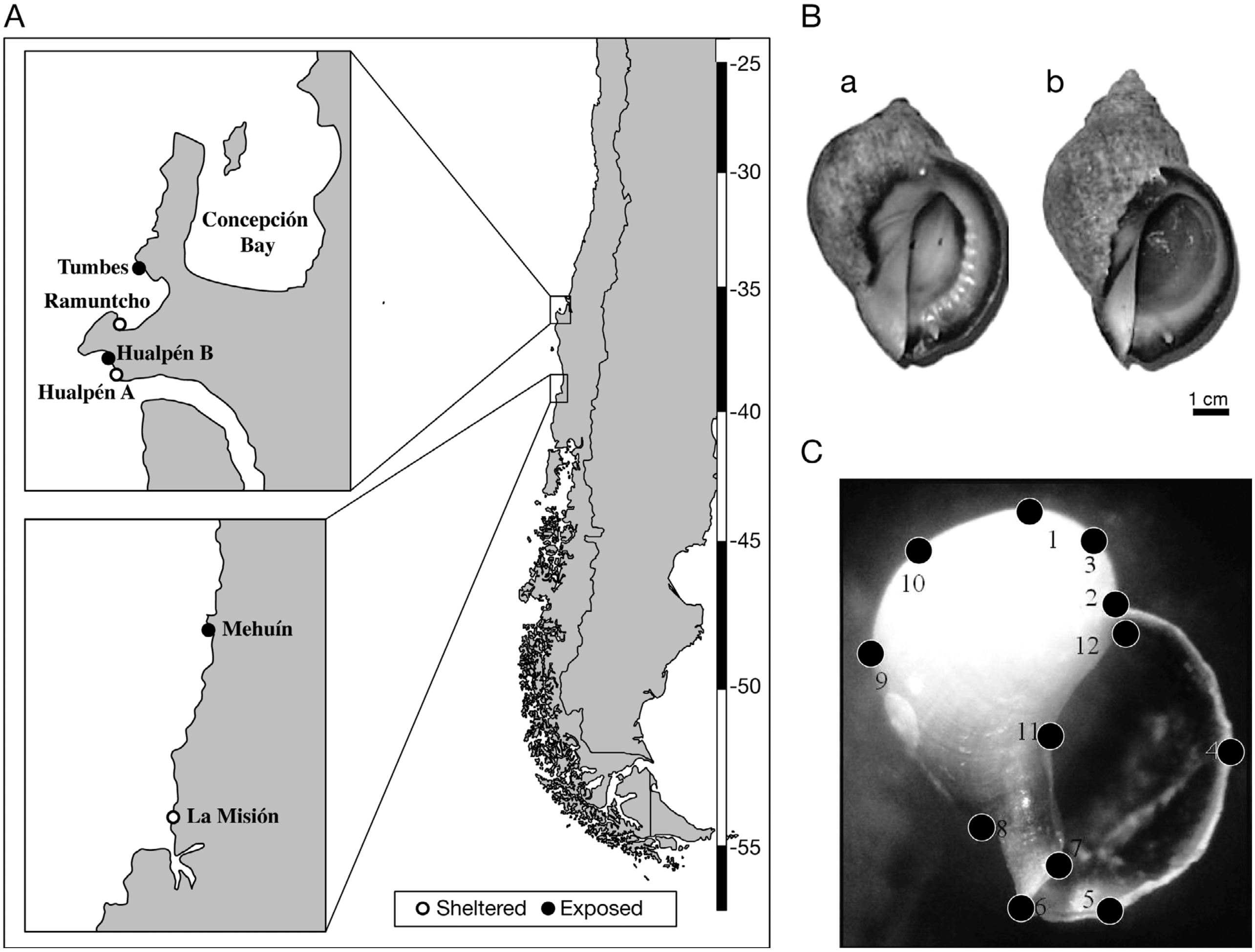

Fig. 1. (A) Location of the wave-exposed and -sheltered sites along the central Chilean coast. Wave-sheltered sites: Hualpén A, Ramuntcho and La Misión; wave-exposed sites: Hualpén B, Tumbes and Mehuín. (B) Morphological types of *Acanthina monodon* adult shells in (a) wave-sheltered and (b) wave-exposed sites. (C) Morphological landmark positions used on the ventral aspect of *A. monodon* shell pictures

and *N. lapillus*, heritability of the shape and size of the shell have been estimated to be between 0.4 and 0.7 (Johannesson & Johannesson 1996, Carballo et al. 2001, Conde-Padín et al. 2007a). Despite the importance of the study of shell morphology in gastropods in understanding adaptive and speciation processes, few studies have been conducted with this group, which makes it difficult to determine the more general mechanisms and patterns that explain diversity.

The marine gastropod *Acanthina monodon* (Pallas, 1774) is a muricid species endemic to the southeastern Pacific Ocean, inhabiting intertidal rocky shores and the shallow subtidal from 28 to 55° S (Gallardo 1979, Valdovinos 1999). *A. monodon* is dioecious with internal fertilization; females enclose their offspring in capsules attached to hard substrata. This species has direct development and juveniles of approximately 0.82 to 1.3 mm hatch from the capsules after 60 to 80 d of intracapsular development (Gallardo 1979). Thus, in the absence of a pelagic larva, the potential for dispersal in *A. monodon* appears to be very low, and, consequently, it has a high level of population genetic structure (Sánchez et al. 2011). Moreover, 2 genetic phylogeographic breaks were observed at 30° S and between 41 and 53° S defining a northern, central and southern phylogeographic area. This species exhibits a high degree of variation in shape, colour and thickness of the shell within and between phylogeographic areas, suggesting local adaptations. Previous studies have suggested that shell morphology in this species would be an adaptive response to predation risk and the degree of wave exposure varying at latitudinal and local spatial scales (Sánchez et al. 2011, Sepúlveda & Ibáñez 2012, Sepúlveda et al. 2012). For example, Sánchez et al. (2011) and Sepúlveda & Ibáñez (2012) found that adult individuals coming from sheltered inner channels in the south of Chile show longer spires, thinner shells and ornamented lips, compared to individuals from the northern sites. In addition, at a local scale, adults from localities with different wave exposure strengths and separated by only a couple of kilometres presented morphological differences, with a larger opercular area at the most exposed site (Fig. 1B). According to preliminary results, such as those reported for *Nucella lapillus* (Kitching et al. 1966; Etter 1988), the increase in the opercular area in *A. monodon* is related to the larger surface of the foot, which would favour attachment strength (A. Barrera pers. comm.). Given the low dispersal potential of *A. monodon* and the adaptive value of shell morphology, it is expected that selection would favour local adaptations with high levels of heritability. In addition, if shell morphological differences observed in adults of *A. monodon* between exposed and sheltered sites do not persist in pre-hatching offspring, this would indicate that plasticity appears to be the main mechanism responding to wave exposition. In this study, using a geometric morphometrics approach, we estimated shell morphology heritability in *A. monodon* with a full sibling design using pre-hatching juveniles in 6 localities with different wave strengths, and we compared size and shell morphology of offspring between sites with high and low wave exposition.

MATERIALS AND METHODS

Study area and sampling collection

During spring 2010 we collected capsules containing pre-hatching juveniles of *Acanthina monodon* from 6 intertidal rocky shores on the Chilean coast: 3 wave-sheltered sites: Hualpén-Sheltered (36° 48′ 23.07″ S, 73° 10′ 36.17″ W), Ramuntcho (36° 47′ 12.60″ S, 73° 11′ 12.55″ W) and La Misión (39° 48′ 45.42″ S, 73° 24′ 1.65″ W), and 3 wave-exposed sites: Hualpén-Exposed (36° 48′ 17.12″ S, 73° 10′ 45.68″ W), Tumbes (36° 43′ 04.96″ S, 73° 07′ 20.34″ W) and Mehuín (39° 25′ 31.29″ S, 73° 13′ 00.18″ W; Fig. 1A). All sites were located in the central phylogeographic area (Sánchez et al. 2011) in order to reduce potential differences or bias in heritability patterns between exposed and sheltered sites due to population genetic structure. To determine the exposure levels at different sites, we used spheres made of dental resin of approximately 240 g each placed in the intertidal. Exposure was determined as a function of degradation of the spheres after 48 h of exposure to the wave action in the intertidal zone where the capsules were collected.

In each site we sampled between 8 and 12 different capsule clutches (hereafter ‘families’), and 3 capsules at pre-hatching stage were collected per clutch. In the laboratory, capsules were dissected under a binocular microscope, and the pre-hatching juveniles were stored in ethanol for posterior analyses.

Morphometric analysis

A geometric morphometric analysis was conducted to estimate the morphological variation observed in *Acanthina monodon* between sampled sites. We employed a geometric model, used in other studies for shell morphology analyses (e.g. Conde-Padín et al. 2007b), that studies the shape of shells using morphological coordinates (landmarks). This method examines the covariation between shape variables among individuals and considers the superimposition of 2 biologically homologous landmark coordinates (LM) that are projected onto a common tangent space and over which the shape variables are obtained free of rotation, translation and scale effects (Rohlf & Slice 1990, Bookstein 1991, Rohlf & Marcus 1993). These variables are then used to generate a statistical analysis and spatial representation of the shape and its variation. Details on how to perform linear statistical models in geometric morphometrics can be found in Dryden & Mardia (1998), Rohlf (1998, 1999) and Klingenberg & Monteiro (2005).

Digital images of the ventral side of the specimens were taken from the shells of pre-hatching juveniles at each study site, using a digital camera (Samsung ST65 14.2MP). On each image, 12 landmarks were digitalized: 7 Type I (homologous anatomical structures) and 5 Type II (maximum curvatures) landmarks (sensu Bookstein 1991) using tpsDig2 software (Rohlf 2005a; Fig. 1C). Similar landmarks were used by Sánchez et al. (2011) and Sepúlveda & Ibáñez (2012). Subsequently, landmarks were transformed into x–y coordinates which were used to perform the statistical analysis. Additionally, we estimated individual centroid size and population consensus configuration, which were used as a measure of linear covariation and average population configuration, respectively. Generalized procrustes analysis (GPA) was performed to remove the variation not attributable to form (Rohlf 1999). GPA is a necessary procedure because it removes variation in digitalizing location, orientation and scale and superimposes the objects in a common (although arbitrary) coordinate system. Additionally, the aligned specimens from GPA provide points that can be projected onto a Euclidean space tangent to Kendall's shape space (Rohlf & Slice 1990, Bookstein 1991, Rohlf 1999, Slice 2001, Adams et al. 2004). The new Cartesian coordinates obtained after superimposition were the shape coordinates used for inter-individual statistical comparisons. The shape differences between landmark configurations of 2 individuals can be summarized by their procrustes distance, which is the square root of the sum of squared distances between pairs of corresponding landmarks. For a review, an introduction to the applications of geometric morphometrics in biology is provided by Rohlf (2005b). To obtain optimal shape variables (i.e. relative warps, Rw), the aligned specimens were compared through tpsRelw (Rohlf 2005c) using $\alpha = 1$, which weights the most remote landmarks producing a global deformation effect in the snails. All Rws are orthogonal components and, therefore, contain independent variance, analogous to a principal component analysis. Finally, centroid sizes (CS) were used as a proxy for the size of individuals; these were determined using tpsRelw (Rohlf 2005c). CS corresponds to a dispersion measure of landmarks around the centroid and are computed as the square root of the sum of squared distances of all landmarks from the centroid.

Heritability in shell morphology

The variation of traits of *Acanthina monodon* shells was obtained by partitioning the variance into 2 components through MODICOS software (Carvajal-Rodríguez & Rodríguez 2005): between (Vf^2) and within (Vo^2) families, assuming that the pre-hatching juveniles from the same group of capsules are full-siblings (Carballo et al. 2001). Thus, we calculated heritability (h^2) as follows:

$$h^2 = 2 \times Vf^2 / (Vf^2 + Vo^2) \qquad (1)$$

This design provides estimations of heritability in a narrow sense. The use of 3 individuals per family allows accurate heritability estimation, with values around 0.6 (Falconer & Mackay 1996). Therefore, in this study, we used 3 juvenile shells per family to estimate the morphological heritability of both shape and size in the *A. monodon* shells.

Statistical analysis

The degradation values of the resin spheres exposed at different sites were transformed to percentage data. Then, these data were compared through 1-way ANOVA using permutations (1-way PERMANOVA) between wave-sheltered and wave-exposed sites. In the same way, we used 1-way PERMANOVA to test the heritability values between wave exposure levels. To compare the morphological shell variation within and between wave exposure levels, only the first 3 Rws, which explained 72.62% of total variance, were used as shape variables. Since there was no correlation between Rw and CS, we performed a nested-PERMANOVA design on the CS variable and on each Rw variable separately using 'exposure' as the fixed factor, 'site' as the random factor nested in exposure and 'family' as the random factor nested in site (Quinn & Keough 2002). Principal components analysis (variance–covariance matrix) was performed on the first 2 shape variables with the aim of representing the average morphological variation of sites within wave exposure levels (Quinn & Keough 2002). All probability values (p_{perm}) were derived from a pseudo-F distribution calculated through 10 000 permutations of the same pooled data; when the simulated permutations were <1000, the probability value was obtained through Monte-Carlo simulations (p_{MC}). All analyses were performed using PERMANOVA+ for the PRIMER statistical package (Anderson et al. 2008).

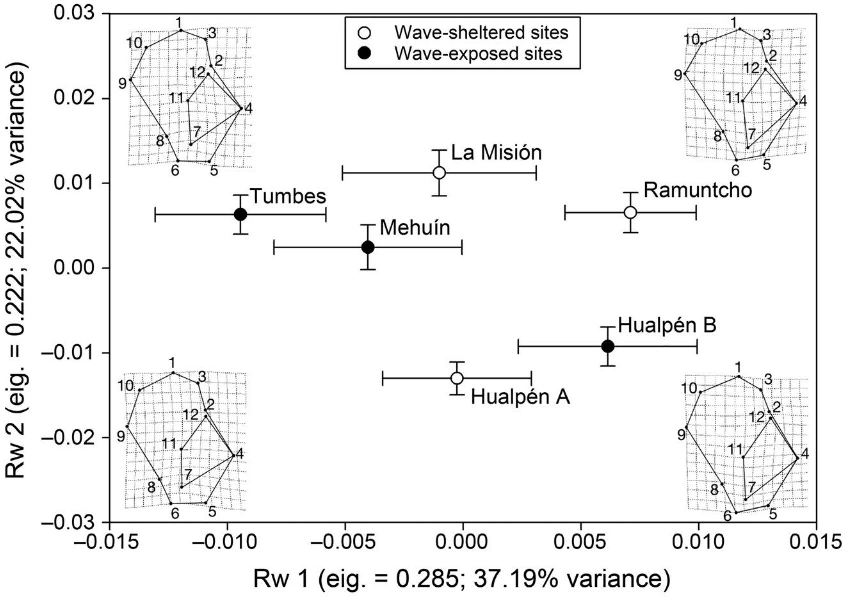

Fig. 2. *Acanthina monodon.* Principal components analysis using average relative warp (Rw) scores (±1 SE) as variables of the *A. monodon* shell shape variation along the central Chilean coast between wave-sheltered and wave-exposed sites. Thin-plate spline representations of snail shells are included for extremes of the gradient for the first 2 Rws. Some landmarks are connected by lines to facilitate interpretation of the differences

Table 1. Results of the morphological analysis (F_{pseudo} values) based on nested-PERMANOVA for centroid size (CS) and for the first 3 relative warps (Rw) in *Acanthina monodon* shells, using 'exposure' as a fixed factor, 'site' as a random factor nested in exposure, and 'family' as a random factor nested in site. $^{*}p_{perm} < 0.05$; $^{**}p_{perm} < 0.01$; $^{***}p_{perm} < 0.001$

Source of variation	df	CS	Rw 1	Rw 2	Rw 3
Exposure	1,4	0.04	0.69	0.04	0.04
Site(Exposure)	4,58	55.00^{***}	3.05^{*}	16.44^{***}	5.56^{**}
Family(Site)	58,127	2.78^{***}	1.27	1.49^{*}	1.67^{**}

RESULTS

Morphological variation in shells

The exposure level, measured as the percentage (±SE) of degradation of the resin sphere, showed significant differences (ANOVA, $F_{pseudo(1,4)} = 13.733$, $p_{MC} = 0.017$) between wave-sheltered (31.5 ± 9.8%) and wave-exposed sites (81.5 ± 9.3%). The morphometric analysis of shells indicated that landmarks related to lip thickness (intersection between outer and inner lip) exhibited the most variation in the analysis (i.e. LM 2 and LM 12; Fig. 1C). Analysis of landmark configurations indicated that landmarks related to lip thickness (LM 2, eigenvector = 88.99) and siphonal channel (LM 7, eigenvector = −105.23) showed greater variations on the *y*-plane of shells in the first Rw. For the second Rw, the same landmarks (LM2: eigenvector = 69.42; LM 7: eigenvector = 66.76) exhibited higher variation on the *x*-plane of the shell (see thin-plate spline, in Fig. 2). Neither shell shape values nor body size (CS) showed significant differences between exposure levels for any of the Rw variables (Table 1, see also Fig. 2 for average morphological variation between and within wave exposure levels). However, as a general pattern, significant differences were observed between sites and families nested in site for both parameters (Table 1). Only Rw 1 was not significant between families (within sites), which means that shell morphology is similar in individuals from the same locality (Table 1).

Heritability in snail shells

Shape heritability was higher at wave-sheltered than at wave-exposed sites for all Rws analysed, averaging (SE) 0.37 (±0.12) and 0.27 (±0.03), respectively (Table 2). However, the ANOVAs did not indicate significant differences between exposition levels for Rw 1 (ANOVA, $F_{pseudo(1,4)} = 0.229$, $p_{MC} = 0.656$), Rw 2 (ANOVA, $F_{pseudo(1,4)} = 0.028$, $p_{MC} = 0.875$), or Rw

Table 2. *Acanthina monodon.* Heritability estimations for centroid size (CS) and for the 3 first relative warps (Rw) in shells between wave-sheltered (Ra: Ramuntcho; Mi: La Misión; Hu-A: Hualpén sheltered) and wave-exposed (Tu: Tumbes; Hu-B: Hualpén exposed; Me: Mehuín) sites. Values in brackets correspond to 1 SE

	Wave-sheltered sites				Wave-exposed sites			
	Hu-A	Ra	Mi	Average heritability	Hu-B	Tu	Me	Average heritability
Rw 1 (37.19 %)	0.3	0.2	0.8	0.43 (0.19)	0.2	0.0	0.7	0.30 (0.21)
Rw 2 (22.02 %)	1.0	0.0	0.0	0.33 (0.33)	0.0	0.7	0.1	0.27 (0.22)
Rw 3 (13.41 %)	0.6	0.3	0.1	0.33 (0.15)	0.3	0.1	0	0.13 (0.09)
Shape heritability	0.63 (0.20)	0.17 (0.09)	0.30 (0.25)	0.37 (0.14)	0.17 (0.09)	0.27 (0.22)	0.27 (0.22)	0.23 (0.03)
CS heritability	0.2	0.8	0.3	0.43 (0.19)	0.5	0.2	0.7	0.47 (0.15)

3 (ANOVA, $F_{\text{pseudo}(1,4)} = 1.385$, $p_{MC} = 0.308$). Similarly, average heritability of shell shape estimated on the 3 Rws showed no differences between wave exposure levels (ANOVA, $F_{\text{pseudo}(1,4)} = 4.383$, $p_{MC} = 0.100$). Shell size heritability was higher but not significantly different at wave-exposed than at wave-sheltered sites, averaging (±SE) 0.47 ± 0.15 and 0.43 ± 0.19, respectively (Table 2; ANOVA, $F_{\text{pseudo}(1,4)} = 0.020$, $p_{MC} = 0.895$).

DISCUSSION

Results showed that *Acanthina monodon* shell morphology is highly variable. Heritability estimations were compared to those of other marine gastropod species with low dispersal. Average heritability for size and shape of shells showed uneven values among sites, oscillating between 0.2 and 0.6 for shape and between 0.2 and 0.8 for shell size. These values are consistent with the observed heritability in many quantitative traits of other molluscs (Falconer & Mackay 1996, Carballo et al. 2001, Conde-Padín et al. 2007a). The heritability of shell morphology in *A. monodon* is in agreement with the expectation that phenotypic variation in low dispersal species is based to an appreciable extent on genetics. In contrast to previous observations where differences were observed in shell shape of adult individuals from sites with different wave strengths, morphology and size of shells of pre-hatching juveniles did not differ between exposed and sheltered sites. This suggests that shell morphology diversity in adults of *A. monodon* could be explained by a more complex interaction between plastic and heritable responses.

Heritability levels observed in *Acanthina monodon* suggest a genetic component in shell morphology. Among localities, heritability was highly variable, fluctuating between 0.2 and 0.6 for shape and 0.2 and 0.8 for size. Heritability between exposure conditions ranged between 0.37 and 0.27 for sheltered and wave exposed sites, respectively. Our estimations are in accordance with several studies on direct-developing gastropod species of the genus *Littorina* and *Nucella* among which shell shape heritability oscillated between 0.4 and 0.7, indicating a high adaptive potential (Boulding & Hay 1993, Johannesson & Johannesson 1996, Carballo et al. 2001, Conde-Padín et al. 2007a). *A. monodon* is also a marine gastropod with direct development and, hence, with a low potential for dispersal, which would favour local selection processes (Sánchez et al. 2011).

Local effects on shell morphology and size have been repeatedly reported in the literature for gastropod species. For instance, Johannesson et al. (1993) found that the high variability in the shell colour of *Littorina saxatilis* individuals was determined by the substrate type at local habitats. Similarly, Manríquez et al. (2009) found that the shell colouration in the muricid *Concholepas concholepas* depended on the prey-substrate used to induce metamorphosis and for post-metamorphic rearing. For morphological traits, variations in the shells of *Thais lamellosa*, *L. saxatilis* and *Nucella lapillus* have been related to different levels of predation where shell thickening is an adaptive response reducing vulnerability to predation (Kirby et al. 1994, Guerra-Varela et al. 2009, Hollander & Butling 2010). In *N. lapillus* 2 ecotypes have been described based on the wave exposure level: the ecotype at exposed sites has a more globular shell, with relatively larger apertures and thinner shells compared to the ecotype at sheltered sites (Kitching et al. 1966). These morphological differences could be explained by adaptations that increase foot surface and thus attachment strength (Etter 1988, Kitching et al. 1966). In *Acanthina monodon*, similar shell morphology variation has been described in the literature, and several reports suggest local adaptations to environmental factors such as predation and wave exposure level (Sánchez et al. 2011, Sepúlveda & Ibáñez 2012, Sepúlveda et al. 2012). As a general pattern, in this work, we have shown that shell morphology and size of pre-hatching individuals varied between sites and family. In spite of the differences observed in shell morphology and heritability, no significant effect of wave exposure level was detected. This means that the high variation in shell morphology observed at the adult stage among sites with different wave strengths could be explained primarily by plasticity, which may suggest that, although a genetic component of shell morphology exists, shell characteristics of *A. monodon* change through ontogeny according to environmental conditions. In addition, a methodological improvement may be suggested to increase accuracy estimations of heritability. We used 3 individuals per family to estimate heritability, which allows detection of significant heritability values around 0.6. It is suggested that by increasing family size the heritability estimates would become more robust and accurate, unmasking potential underlying patterns between localities and wave exposure conditions.

The extreme-phenotype hypothesis suggests that a phenotypically plastic response might only be able to produce a less extreme phenotype, compared to a phenotype previously adapted to the same environment, due to the costs of possessing a plastic response or constraints on the ability of plastic development to achieve the optimal phenotype. For instance, reciprocal transplants of pre-hatching juveniles of *Littorina saxatilis* have demonstrated that, although plasticity is observed in shell morphology in response to local environmental conditions, juvenile individuals maintain the imprinting induced by their native habitats, exhibiting higher mortalities compared to individuals native to the habitat (Hollander & Butling 2010). In *Acanthina monodon*, we do not know if this kind of a response exists; however, we can expect that, given the low dispersal potential of the species and the genetic component and adaptive value of shell morphology, potential constraints in phenotypic plasticity may be observed in response to local adaptations. It is clear that reciprocal transplants of individuals between contrasting wave exposure conditions are needed to determine the adaptive value of phenotypic plasticity in *A. monodon*.

The heritability estimations using full-siblings do not only include heritability in a narrow sense, but potentially also a component due to dominant or common environmental effects (Falconer & Mackay 1996). This means that if the loci contributing to shell measurements show common dominance components, the estimated heritability would be inflated. Alternatively, other factors may influence heritability estimations. The assumption that pre-hatching juveniles are full-siblings may be inaccurate, as some marine gastropods present multiple paternities within capsules, producing half-sibling capsule mates (e.g. Brante et al. 2011). If multiple paternity exists in *Acanthina monodon*, our estimations of the shell morphology heritability would be very conservative (Guerra-Varela et al. 2009). Maternal effects, such as nutrients delivery or micro-environmental conditions, may also influence heritability estimations (Falconer & Mackay 1996). However, laboratory studies performed with other gastropod species suggest that maternal and microenvironmental effects common to family members do not have a major influence (Carballo et al. 2001; but see Pascoal et al. 2012). It is clear that controlled experiments are needed to determine the potential effects of multiple paternity and maternal conditions on heritability estimations.

Results obtained for *Acanthina monodon* in this study and those observed in other marine gastropod species with a low dispersal potential suggest that shell morphology is an adaptive trait with an important genetic basis and a plastic potential. *A. monodon* is an interesting biological model for studying adaptive and selective local processes and for understanding the causes and mechanisms of phenotypic variation evolution in marine gastropod species. Comparisons with species with a higher dispersal potential and exposed to higher environmental uncertainty during ontogeny would shed more light on the role of life history strategies on phenotypic adaptation.

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