

Field heritabilities and lack of correlation of snail shell form and anti-predator function estimated using Bayesian and maximum likelihood methods

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ABSTRACT

Problem: It is often assumed that the heritability of performance traits (function) can be estimated by the heritability of primary traits (e.g. form). It is also frequently assumed that performance traits should be less heritable than primary traits. These assumptions should be carefully evaluated rather than assumed. One common area of study in which these assumptions have been made is anti-predator traits and performance.

Organisms: A freshwater aquatic snail species, the Mexican banded spring snail *Mexipyrghus churinceanus*, endemic to the isolated Cuatro Ciénegas valley in Mexico. The crushing predator of this snail species, the fish *Herichthys minckleyi*, is also endemic to the valley. We studied the free-ranging snail population.

Methods: We estimated narrow-sense heritability of shell thickness and shape (form), and crushing resistance (function) under field conditions. We used multi-locus genotyping to reconstruct a pedigree in a small wild population, and used animal models to estimate genetic and environmental variance components needed to estimate heritabilities. We estimated variance components using Bayesian inferences and also, for comparison, the traditional restricted maximum likelihood approach. We also estimated pairwise phenotypic correlations between traits.

Results: The two methods produced similar results, although the maximum likelihood approach was more conservative. The trait closest to fitness (crushing resistance) exhibited the greatest heritability: the heritability of crushing resistance was at least twice as high as the heritability of thickness and shape. No phenotypic correlations were evident between traits.

Conclusion: The heritability of form traits (either shell shape or thickness) was not a suitable surrogate for the heritability function (shell crushing resistance). Another assumption found untenable in this study is that traits closer to fitness should have lower heritability. In this study, function was more heritable than form.

Keywords: animal model, anti-predator traits, Bayesian Markov Chain Monte Carlo, form → function → fitness, geometric morphometrics, quantitative genetics, response to selection, restricted maximum likelihood.

INTRODUCTION

Form traits (e.g. morphology) determine aspects of function (performance capacity) that in turn determine fitness (Arnold, 1983; Johnson *et al.*, 2008). Given the expectation of a straightforward relationship between form, function, and fitness, it is often assumed that measuring heritability of form traits provides an acceptable means to estimate the response of those traits to selection. Another common assumption in evolutionary genetics is that trait function should exhibit lower heritability than trait form, because function is expected to be parsed more strongly, and more directly, by selection, which would then most strongly reduce genetic variance in function traits relative to form traits (Falconer and Mackay, 1996; Geber and Griffen, 2003; but see Houle, 1992; Merilä and Sheldon, 1999). We are not aware of studies that may have tested for heritabilities of both form and function, and addressed their relative magnitude, which leaves a lot assumed rather than verified. Such research would seem simple in any of the classic model systems in which form and function have been intensively studied.

Shelled molluscs are excellent and traditional models to study the evolution of morphological traits and anti-predator performance (Vermeij, 1993). Several studies have shown that shell form (i.e. shape, thickness, composition) impacts resistance to predation (function). Molluscs experience four primary modes of predation: whole-shell swallowing, as well as shell crushing, entry, and drilling (Vermeij, 1993; DeWitt *et al.*, 2000). In the case of shell crushing, for example, resistance to predation is determined by combinations of shell thickness, size, composition of shell material, and shell shape (Appleton and Palmer, 1988; Trussell, 1996; West, 1996; DeWitt *et al.*, 2000; Avery and Etter, 2006; Lakowitz *et al.*, 2008; Bourdeau, 2010). To the extent these form traits are heritable and contribute to anti-predator function, they should respond to selection.

Although heritability of shell performance (e.g. crushing resistance) has not, to our knowledge, been estimated in any species, aspects of shell form when assayed are generally found to be heritable (Boulding and Hay, 1993; Conde-Padin *et al.*, 2007; Guerra-Varela *et al.*, 2009). Selection is also typically measured on shell primary traits, rather than on function *per se* (DeWitt and Langerhans, 2003; Boulding *et al.*, 2007). The response to selection is directly proportional to selection differentials and heritability (Falconer and Mackay, 1996). Therefore, measures of heritability on primary form traits would be expected to indicate evolvability only in cases where heritabilities of these traits are similar in magnitude and/or correlated to the heritability of performance traits like crushing resistance. If one estimates heritability of a single trait thought to be related to crushing resistance, predicting the response to selection by shell-crushing predators may be flawed. To our knowledge, the heritabilities of crushing resistance, shell shape, and shell thickness have not been simultaneously estimated in any species. Here we estimate the heritability of these form and function traits in a freshwater snail that co-exists with a shell-crushing fish but with no other predators.

Shell crushing is the sole known mode of predation experienced by our study species, the small (< 8 mm long) Mexican banded spring snail, *Mexipyrghus churinceanus* (Hydrobiidae) (Hulsey *et al.*, 2005, 2006; Johnson *et al.*, 2007; Chaves-Campos *et al.*, 2011). This snail species is only found in soft sediments of hot and saline springs and freshwater streams in the small Cuatro Ciénegas valley (~1500 km²), located in Coahuila, Mexico (Hershler, 1985). This snail co-exists with a crushing predator that is also endemic to this valley: the fish *Herichthys minckleyi* (Chaves-Campos *et al.*, 2011). The snail has a durable strong shell, which, however, can be crushed by this fish (Hulsey *et al.*, 2005). Only snails with stronger shells survive predation once captured by a fish (Hulsey *et al.*, 2005, 2006). Snails that are too hard to be crushed pass through the digestive system of the fish alive, thanks to the presence of an operculum that isolates them from digestive enzymes (Hulsey *et al.*, 2005, 2006). Overall, this characteristic suggests that crushing resistance is at least a primary determinant of fitness in this species.

We estimated the heritability of crushing resistance and form traits from field samples. We have been unable to reproduce the snail's environment well enough in captivity to stimulate breeding with the goal of estimating quantitative genetic parameters under controlled conditions. However, we were able to use genetic markers on field samples to estimate such parameters. We used individual-based mixed models, 'animal models', that use pedigrees to estimate quantitative genetic parameters in wild populations (DiBattista *et al.*, 2009). Animal models are more powerful than traditional methods because they use all possible relationships in a pedigree (Kruuk *et al.*, 2008). We reconstructed the pedigree using recent methods based on molecular markers (Jones and Wang, 2010).

Quantitative genetic parameters are usually estimated with animal models via restricted maximum likelihood (REML), whereas Bayesian approaches have been relatively underexploited in evolutionary biology (Kruuk, 2004; Kruuk *et al.*, 2008). For this reason, comparisons between methods are rare in ecological and evolutionary literature, especially in free-ranging populations (for an exception, see Charmantier *et al.*, 2011). We use both REML traditional approaches and Bayesian approaches for comparison. To the best of our knowledge, this is one of the first applications of the animal model to a free-ranging invertebrate population.

METHODS

Field procedures

We collected the snails from a single natural small pool (16 m in diameter) to maximize the possibility of obtaining related individuals in our field sample. The pool was located in the Pozas Azules reserve (26°49.830N, 102°01.760W) (Coghill *et al.*, 2011). We collected the snails systematically, in July 2009, by setting three parallel transects separated by 4 m from each other that crossed the pool from side to side. We collected snails in quadrats set every 3 m in each transect using an 18 cm square frame placed on the substratum. Sediment down to 5 cm deep within each quadrat was collected with a dip net, and snails were separated from sediment under a dissection microscope fitted with a camera. In total, 82 snails were collected from the 12 quadrats sampled. Shells were placed aperture down and photographed at a resolution of 100 pixels per millimetre. We measured shell length and body whorl (i.e. the largest and most recently formed whorl) width from the pictures (Fig. 1) using the program tpsDig 2.14 (Rohlf, 2009).

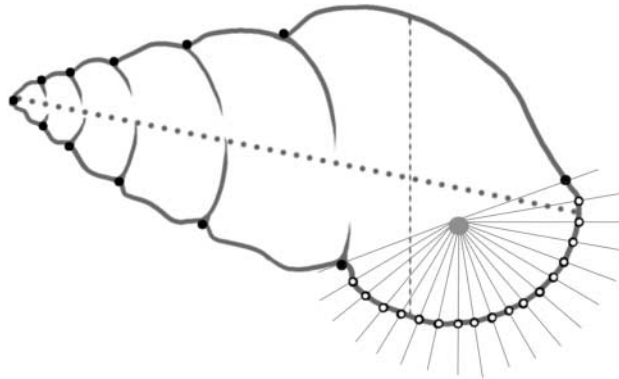


Fig. 1. Landmarks and morphological measures taken from pictures of *Mexipyrigus churinceanus* shells. Shell length was measured as the longest distance between the apex and the outer lip (dotted line). Shell width was measured as maximum body whorl width (dashed line). Landmarks were placed on whorl sutures (solid symbols) and semi-landmarks on the aperture lip (open symbols).

Laboratory procedures

We measured crushing resistance in the laboratory following a method designed to simulate the way in which the fish predator crushes the snails. The predator fish *H. minckleyi*, has pharyngeal jaws – modified gill arches in the throat – used to crush snails (Hulsey *et al.*, 2005, 2006). Experimental studies show that the fish compresses the shell between the pharyngeal jaws until the shell collapses, a process that can be mimicked in the laboratory using the two plates of a force gauge (Hulsey *et al.*, 2005). We used this method to measure the force in Newtons needed to crush each individual snail at the time of shell failure. In short, snails were crushed between two force plates of a Chatillon DFM50 force gauge with an automated Chatillon LTMCM-6 stand as described in Chaves-Campos *et al.* (2011). A shell fragment from the mid part of the body whorl was photographed under maximum magnification (250 pixels per millimetre) to measure the thickness of each shell (in transverse view) using tpsDig. We were able to measure shell thickness in a standard way for 66 snails. Foot tissue from all snails was preserved in 95% ethanol for DNA extraction.

Morphometric analyses

Because shell-crushing resistance increases with shell length in *M. churinceanus* (Hulsey *et al.*, 2005; Chaves-Campos *et al.*, 2011), we described the relationship between these two variables using linear regression and used the regression residuals as descriptors of size-adjusted crushing resistance (Chaves-Campos *et al.*, 2012) to estimate quantitative genetic parameters. We also estimated size-adjusted crushing resistance values using shell centroid size for comparison. The two analyses produced similar results, so we only report results from the analysis using shell length. Shell thickness was not significantly affected by shell length, so we used unadjusted values in the animal models.

We described shell shape using both ‘traditional’ and geometric morphometric methods. For traditional morphometrics, we described shape using shell length and width (i.e. body whorl width), as these two variables can roughly describe the rotundness of snail shells (DeWitt *et al.*, 2000). We described the relationship using principal components analysis (PCA).

The first and second principal components (PC1, PC2) represented body size and shape, respectively (see Table S1 at evolutionary-ecology.com/data/2787Appendix.pdf), so we used PC2 as an index of shape in the heritability analysis. For geometric morphometric analysis, we digitized 28 points along the outline of the shell using tpsDig. Of these, 10 were considered landmarks that corresponded to whorl sutures, while the rest corresponded to semi-landmarks on the shell outer lip (Langerhans and DeWitt, 2002) (Fig. 1). Only snails with six whorls were included in the geometric morphometric analysis; three small snails with only five whorls were excluded. Semi-landmarks were slid with respect to adjacent landmarks/semi-landmarks to reduce their influence during superimposition (Bookstein, 1997). This method is a fix for the dimensional deficiency of semi-landmarks, coded each with 2 bits of data, when in fact they only possess one degree of freedom (length of the transect that defines them). Once semi-landmarks were slid, they were superimposed with the full landmark suite and entered into the PCA. Superimposition, semi-landmark sliding, and PCA were all conducted with the program TpsRelw 1.49 (Rohlf, 2010). The first 22 principal components explained 99% of the variation in shape and were used in a multivariate analysis of heritability (Klingenberg and Monteiro, 2005) (see Table S2 at evolutionary-ecology.com/data/2787Appendix.pdf). The results obtained with PC1 were similar to those for the other 21 principal components, so we only show heritability estimates for this principal component in the results. PC1 alone explained 48% of the variance in shape, specifically variation in relative aperture size and apex angle (Fig. 2).

Pedigree reconstruction

A multi-generational pedigree was constructed using genotypes from eight species-specific microsatellite markers (Coghill *et al.*, 2011). PCR conditions were as described in Coghill *et al.* (2011). The program Colony 2.0.1.1 (Jones and Wang, 2010) was used to reconstruct the genetic relationships among individuals in the sample, which allowed us to reconstruct the pedigree. We genotyped 15–20% of the individuals twice at each locus for quality control without detecting genotyping errors. Nevertheless, we ran the program assuming 1% error. The pedigree is presented in Table S3 (see evolutionary-ecology.com/data/2787Appendix.pdf).

Heritability estimates using Bayesian inference

The R package MCMCglmm (Hadfield, 2010) was used to estimate the narrow-sense heritability [i.e. the proportion of phenotypic variance due to additive genetic effects (Falconer and Mackay, 1996)] of shell-crushing resistance, thickness, and shape. This method employs an animal model to estimate variance components through a Bayesian Markov Chain Monte Carlo (MCMC) approach. We estimated the additive genetic variance, variance due to sampling location (i.e. quadrat) – both considered random effects in the model – and residual environmental variance. No mother–offspring dyads were identified in the sample, which made it impossible to account for potential phenotypic variation due to maternal effects [i.e. the effects of the environment and genotype of the mother on the offspring phenotype (Falconer and Mackay, 1996)]. We fit an animal model for each trait independently (i.e. univariate models) using a Gaussian distribution after confirming that each variable was normally distributed using the Shapiro-Wilk test (not shown).

Bayesian analysis requires the specification of priors. We did not find published information on the heritability of crushing resistance, shape, or thickness for any freshwater

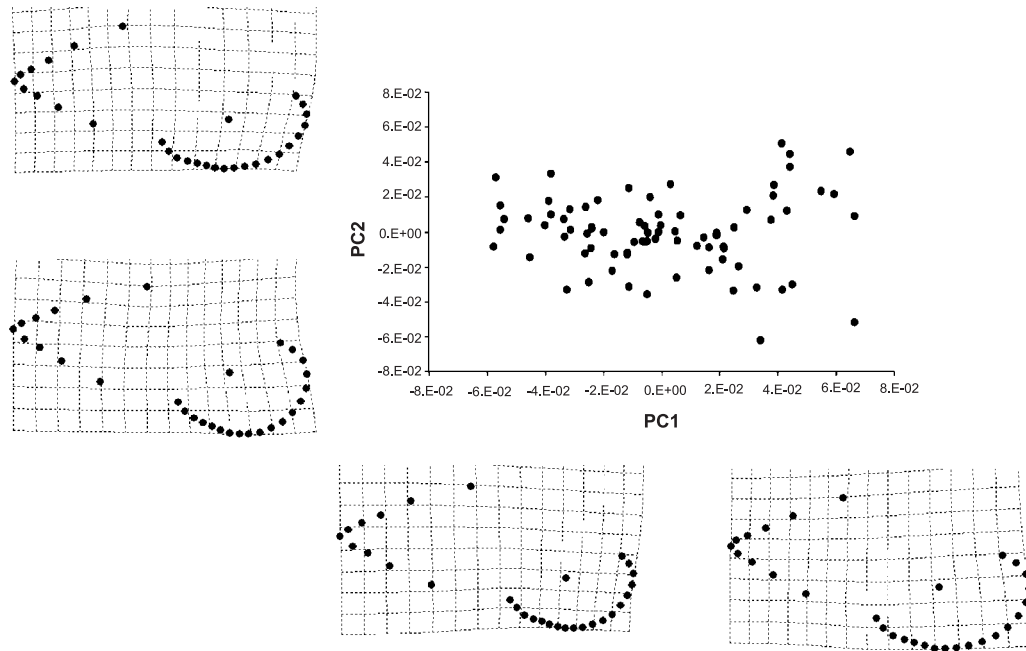


Fig. 2. The first two PCs of a principal components analysis on Procrustes coordinates of the landmarks and semi-landmarks described in Fig 1. Thin plate reconstruction of variability is shown for each principal component for extreme positive and negative values. PC1 explained variation in aperture size and apex angle, ranging from shells with a large aperture and ample apex angle (positive scores) to shells with a narrower aperture and sharper apex angle (negative scores). PC2 explained variation in aperture roundness, with variation ranging from shells with more elliptical apertures (positive scores) to shells with more round apertures (negative scores).

snail species, so we assumed that the contribution of the genetic, random, and residual effects were equivalent when we set the priors. Using priors as high as 0.5 for genetic effects, based on the heritability of shell shape in a marine snail (Conde-Padin *et al.*, 2007), did not change our conclusions. We tested the significance of the additive genetic variance component by running models with and without the ‘animal’ term (i.e. pedigree information) to compare them using the deviance information criteria (DIC) (McFarlane *et al.*, 2011). A difference larger than 7 units is accepted as strong evidence in favour of the model with the smaller or more negative DIC (Spiegelhalter *et al.*, 2002). We used a similar approach to test the significance of the quadrat component.

Heritability estimates using a traditional method

We repeated the analysis using a more traditional approach for comparison. Univariate models were implemented in ASReml-R 3.0 (VSN International; <http://www.vsn-intl.com>) to estimate variance components using REML. The significance of random effects was assessed using traditional frequentist statistics in ASReml-R. Specifically, we compared models that included the animal term with models that did not, using likelihood ratio tests. When the animal term was significant in a model, an additional likelihood ratio test was

conducted between the model that included the animal term with a model that included the animal and the quadrat term to assess the effect of the latter.

Useful estimates of heritability can be obtained from less than a hundred records as in this study, but a much larger sample size is needed to estimate useful genetic correlations (Wilson *et al.*, 2010). For this reason, we did not estimate genetic correlations in this study. Instead, we calculated phenotypic correlations using Pearson correlation coefficients to have an initial idea of the relationships between variables.

RESULTS

Heritability estimates using Bayesian inference

The three traits measured varied greatly among individuals (see Table S4 at evolutionary-ecology.com/data/2787Appendix.pdf). In the case of the MCMC Bayesian approach, models with the animal term always had much smaller or negative DIC than models without this term (Table 1), indicating that the heritability estimates were significantly different from zero (McFarlane *et al.*, 2011). Adding the quadrat term to the model decreased the DIC substantially (i.e. > 7 units) in the case of crushing resistance and shell thickness, resulting in slightly lower heritability estimates (Table 1). Heritability estimates from the final models were low or moderate for shape and thickness. The heritability estimate for crushing resistance was very high; at least twice as high as the others. The 95% highest posterior density intervals (HPDI) were broad in all cases, however, and overlapped between trait estimates.

Heritability estimates using a traditional method

The heritability estimates obtained using REML were slightly higher than the estimates obtained with the MCMC Bayesian approach in the case of thickness and shape. The only exception was that the heritability of shape using geometric morphometric data was much lower. The heritability estimates were very similar with both methods in the case of crushing resistance (Table 1). As in the case of the MCMC Bayesian estimates, the heritability of crushing resistance was at least twice as high as the heritability of thickness and shape (Table 1). Only the heritability of crushing resistance was significantly different from zero using the REML approach (likelihood ratio test: $\chi_1^2 = 4.4$, $P = 0.04$ for crushing resistance; $P > 0.3$ in the other cases). Adding the effect of quadrat to the model that included crushing resistance did not improve the model (likelihood ratio test: $\chi_1^2 = 1.9$, $P = 0.16$), indicating that the effect of quadrat does not contribute to the genetic components of variance. Finally, none of the phenotypic correlations were significantly different from zero (Table 2).

DISCUSSION

Comparison of the MCMC and REML approaches

In general, the two methods produced qualitatively similar results, i.e. the heritability of crushing resistance was much higher than the heritability of shape and thickness, but the REML approach detected fewer significant random effects. Whereas the MCMC method detected a significant genetic variance component for the three shell traits, the REML

Table 1. Narrow-sense heritability estimates (h^2) for shell thickness (TH), crushing resistance (CR), and shape in the snail *Mexiphyrgus churinceanus* estimated either with Bayesian inference or with restricted maximum likelihood (REML)

Model	Bayesian				REML			
	DIC	h^2	95%HPDI	LL	V_A (S.E.)	V_Q (S.E.)	V_R (S.E.)	h^2
TM	-68.20			98.20				
TM + V_A	-89.40	0.20	(0.06-0.81)	98.73	6.5E-3 (7.4E-3)		1.5E-2 (7.1E-3)	0.30
TM + V_A + V_Q	-90.40	0.22	(0.04-0.75)	98.73	6.5E-3 (7.4E-3)	1.5E-9 (7.2E-10)	1.5E-2 (7.1E-3)	0.30
GM	-284.8972			205.04				
GM + V_A	-290.5825	0.17	(0.04-0.63)	205.04	1.1E-9 (1.8E-10)		1.1E-3 (1.8E-4)	1.0E-6
GM + V_A + V_Q	-291.2198	0.08	(0.02-0.55)	205.04	1.1E-9 (1.8E-10)	3.2E-10 (5.4E-11)	1.1E-3 (1.8E-4)	1.0E-6
TH	-226.75			167.94				
TH + V_A	-248.11	0.20	(0.06-0.84)	168.29	4.6E-4 (5.9E-4)		1.1E-3 (5.7E-4)	0.30
TH + V_A + V_Q	-258.23	0.12	(0.03-0.65)	170.66	2.5E-4 (4.9E-4)	4.80-4 (3.7E-4)	9.5E-4 (4.9E-4)	0.15
CR	614.60			-234.17				
CR + V_A	567.60	0.70	(0.18-0.93)	-231.97	108.0 (62.9)		59.0 (50.6)	0.65
CR + V_A + V_Q	554.66	0.67	(0.14-0.87)	-230.99	106.4 (60.3)	10.9 (12.9)	49.2 (48.0)	0.64

Note: Shape was described with traditional (TM) or geometric morphometrics (GM). Models with and without additive genetic variance (V_A) and variance due to the quadrat where snails were collected (V_Q) are shown for comparison. Bayesian models with the smallest deviance information criterion (DIC), at least 7 units apart from the next model, are shown in bold. The 95% highest posterior density interval (HPDI) is shown for Bayesian h^2 estimates. REML models with significantly larger likelihood (LL) according to likelihood ratio tests are shown in bold. The residual variance (V_R) is shown for REML models. Variance components are shown with one standard error (S.E.).

Table 2. Pairwise phenotypic correlation matrix for crushing resistance (CR), thickness (TH), and shape (S) in the snail *Mexipyrghus churinceanus*

	S	CR	TH
Traditional morphometrics			
S		1.00	1.00
CR	0.10		1.00
TH	0.02	0.02	
Geometric morphometrics			
S		0.99	0.12
CR	0.09		0.99
TH	-0.27	0.02	

Note: The second principal component was used in the case of traditional morphometrics, while the first principal component was used in the case of geometric morphometrics (see text). Pearson correlation coefficients are presented below the diagonal. A total of 60 individuals for which all three traits were measured were included in the analyses. Respective *P*-values (adjusted for multiple comparisons) are presented above the diagonal. Unadjusted *P*-values are shown in Table S5 (evolutionary-ecology.com/data/2787Appendix.pdf).

approach only detected a significant genetic component in the case of crushing resistance. Furthermore, whereas the MCMC method detected a significant contribution of the quadrat where snails were collected (at least in the case of models that included crushing resistance and thickness), the REML method did not detect significant contributions of the quadrat term in any model.

Comparisons between Bayesian methods using MCMC algorithms and REML methods using simulated data show that the two approaches produce similar heritability estimates (Wilson *et al.*, 2010) but a recent study using real data indicates that REML notably underestimates heritabilities (Charmantier *et al.*, 2011). The underestimation detected in the latter study for models fit using REML should not be surprising, because this study analysed non-Gaussian data, whereas the simulated data used in the study by Wilson *et al.* (2010) followed a Gaussian distribution. It is known that there are difficulties and uncertainties associated with both parameter estimation and hypothesis testing for non-Gaussian traits using REML methods, whereas Bayesian inference via MCMC offers a way around some of these difficulties (Wilson *et al.*, 2010). Hence, the results obtained using MCMC Bayesian inference should be more robust than results obtained using REML when the data are not normally distributed (Charmantier *et al.*, 2011). In our study, however, the data were normally distributed.

Our results suggest that the REML approach may be more conservative at detecting significant random effects than the MCMC Bayesian approach with Gaussian traits. The conservatism of the overall approach can be attributed to the use of likelihood ratio tests to evaluate the significance of random effects, as these tests are considered overly conservative (Wilson *et al.*, 2010). This potential issue can be circumvented by comparing hierarchical models fit with REML using the standard Akaike's information criterion (Burnham *et al.*, 2011; Charmantier

et al., 2011). We decided to be conservative so we did not pursue the latter approach. In any case, the heritability estimates produced by the two methods are similar in magnitude for the most part with or without the inclusion of the quadrat term in our study (Table 1). More importantly, the relative difference between heritability estimates of form and function traits is the same with both methods.

Heritability of form and function traits

Our results suggest that the heritability of the function trait (crushing resistance) is much higher than for form (i.e. the primary traits thickness and shape). Moreover, the heritability of crushing resistance can be heritable when the heritability of thickness and shape is not. This result contradicts the classical Fisherian expectation that functional traits should have reduced heritability relative to primary traits (Falconer and Mackay, 1996; Geber and Griffen, 2003). This expectation derives from the idea that selection should be stronger on traits closest to fitness, such as functional traits, because selection reduces additive genetic variance (but see Houle, 1992). However, some types of traits, such as life history or even fitness itself, may have higher heritabilities than morphological traits due to a larger number of loci and complicated genetic architecture compared with non-fitness traits (Merilä and Sheldon, 1999).

Our heritability estimates can be affected by environmental and maternal effects, but perhaps not in a way that can change the relative order among traits described above. The MCMC Bayesian analysis suggests that the quadrat where a snail was collected accounts for a small proportion of phenotypic variance in both crushing resistance and thickness, but not in shape. The more conservative REML analysis shows that the effect of quadrat is negligible. The effect of quadrat makes sense in this case because the local abundance of aquatic macrophytes may determine the thickness and quality of material deposited in the shell of *M. churinceanus* (Chaves-Campos *et al.*, 2012), affecting the heritability estimates for thickness and crushing resistance but not shape. Maternal effects are also likely in *M. churinceanus* because females brood offspring in their shell (Hershler, 1985), but could not be measured in this study. Not including maternal effects in the calculation of heritability can result in inflated estimates (Meyer, 1992), but there is no obvious reason to believe that maternal effects would inflate the crushing resistance heritabilities more than they would inflate the other two heritabilities.

In addition, we found no evidence of phenotypic correlation between crushing resistance and either thickness or shape. The lack of significant phenotypic correlations might be taken as an initial indication of a lack of genetic correlations between these traits, because both measurements are usually strongly correlated with each other (Kruuk *et al.*, 2008). Further studies should be conducted to confirm this pattern but the data presented here suggest that crushing resistance may not be genetically correlated with shape and thickness. This should not be surprising because there are other functions that shells perform that are unrelated to crushing resistance. For instance, differences in shell shape and thickness have been related to sexual and fecundity selection in aquatic snails (Cruz *et al.*, 2001; Riascos and Guzman, 2010), casting more doubt on the assumption that the heritability of crushing resistance can be estimated in a straightforward fashion from the heritability of shell shape and thickness.

Overall, our results indicate a potential problem in assuming that the heritability of shell form traits is equivalent to that for shell-crushing resistance. In addition, our results indicate the possibility that using the heritability of either shape or thickness as a surrogate for the heritability of crushing resistance may underestimate the response to selection on

shell-crushing resistance. Hence, resistance to crushing should be measured directly when the evolution of resistance to predation is studied in snails. More generally, these results bring attention to Arnold's paradigm (Arnold, 1983), that the relationship between form and function should be carefully studied before assuming any relationship between form and fitness (Irschick, 2002; Johnson *et al.*, 2008).

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