Multiple scale patterns of shell and anatomy variability in land snails: the case of the Sicilian *Marmorana* (Gastropoda: Pulmonata, Helicidae)

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Certain major aspects of phenotypic diversity are still largely unexplained. When phenotypic patterns do not relate to habitat variables, fine analysis of morphological patterns and their distribution sheds light on the origin of diversity. Among invertebrates, snails are an ideal model for studying the roles of the neutral processes and selection involved in creating diversity. To understand patterns and processes of variability on different scales (regional: areas; local: sites), morphological variability of two sets of characters (shell and genitalia) was quantified in a group of rock-dwelling land snails of the genus *Marmorana* (Pulmonata, Helicidae). To analyse shell variability, partitioning of the overall variation into size and shape components was analysed by a principal component-based approach. Shell shape and size variability is not significantly influenced by any environmental pressure. Variability at site scale is mainly attributed to shell size, which is a trait demonstrated to have a high degree of phenotypic plasticity. No sharp changes were observed for genitalia. Moreover, allometries between shell size and genitalia measurements involve a few populations. The observed multiple scale patterns are in line with the hypothesis that genital variance may be selectively controlled to maintain function. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, 93, 359–370.


INTRODUCTION

Geographic variation has always been a major aspect of evolutionary biology. Attempts to solve the geographical ‘crazy quilt’ distribution of variability (Gould, 1984) have provided insights into the role of neutral and selective processes in the origin of diversity. Terrestrial gastropods are a crucial group of animals for studying these issues (Davison, 2002). Their shells have enabled naturalists to describe more than 25 000 species in the world since the 18th century and to observe variation patterns in the wild. Malacologists began to investigate anatomy in the 19th and 20th centuries, looking for systematically significant characters or evidence of sexual selection. The issue of ‘history versus selection’ in explaining patterns of shell variability (such as shell polychromatism) is investigated in the so-called area-effect studies on *Cepaea nemoralis*: groups of populations are separated from each other by steep gradients of gene frequencies, often where the habitat appears to be uniform (Cain & Currey, 1963). Cain & Currey (1963) attributed area-effects to ‘selection by cryptic features’ of the microclimate. Subsequently, other hypotheses have invoked aspects of population history (e.g. genetic drift or differentiation in refugia) or recent selection (Goodhart, 1963; Clarke, 1966; Cameron, Carter & Palles-Clark, 1980). However, a historical explanation cannot exclude the effect of selection in the past, so that the problem comprises distinguishing between a neutral or selective interpretation (Gould & Woodruff, 1990; Davison & Clarke, 2000). Such discrepancies in *Cepaea* were viewed as a consequence of habitat instability related to human activities or the result of invasions from...
refugia in which genetic differentiation occurred. More recently, Bellido et al. (2002) highlighted the great role of random processes in spatially modelling shell polymorphism in populations of *Cepaea nemoralis*.

The functional aspects of shell morphology in land snails have become of great interest, particularly since the observation of Cain (1977) that shell shape has a bimodal distribution in widely separate and taxonomically distinct land snail faunas. Snails tend to be either high spired or flat, with few globular forms between. Moreover, shell types are often associated with particular habitats (Goodfriend, 1986; Heller, 1987; Cameron & Cook, 1989; Cook, 1997). A good review on the variation of shell shape and size in land snails and its causes is presented by Goodfriend (1986). On the one hand, studies of shell shape variation have involved analysis of the distribution of shapes among taxa and the interpretation of shape frequency differences and, on the other hand, investigation of correlations between shape variation and environmental variation. Studies on shell size, instead, have shown that size differences among populations could be inherited and that within-population size variation may also have a significant genetic component. They have also provided evidence of direct environmental influence on shell size through different variables.

Concerning anatomical variability in land snails, some studies (Davis et al., 1992; Davis, Chen & Yu, 1994; Wilke, Pfenninger & Davis, 2002) demonstrated that unusually high intraspecific variability indicates poor taxonomic resolution of anatomical characters. However, little is known about anatomical variability and its correlation with shell variability or about intra- and interpopulation anatomical variability, although qualitative characters of genitalia are extensively used for taxonomic and systematic studies. Some studies measuring soft body parts recently considered the distinction between size and shape effects and recent evidence of functional relationships between certain parts of the distal genitalia (Lace, 1992; Koene & Chase, 1998; Van Osselaer & Tursch, 2000; Madec, Bellido & Guillier, 2003).

Regarding experimental design, little attention has been paid to the effect of spatial scale on shell and anatomical biometrics (Welter-Schultes, 2001). As a rule, biological variables show spatial and temporal variations. Indeed, on different scales, variables may show different patterns of variability. Thus far, the scenario emerging from the literature focuses on shell variability in a particular malacofauna, or at the species level, but not on a fine scale. This means that little is yet known about possible fluctuations and plasticity in shape and size of shell and anatomy within populations and among populations of different areas.

Despite much research into molecular genetic variation in snail species, major aspects of phenotypic diversity are not yet understood (Davison, 2002). Especially when shell shape patterns do not relate to environmental variables, fine analysis of morphological variability and its distribution could shed light on the origin of diversity.

The present study aimed to quantify morphological variability on regional and local scales, and to attempt a biological interpretation of any regional or local differences in shell and anatomical characters. The subject of this study comprised a group of rock-dwelling globular to flat-shelled land snails belonging to the genus *Marmorana* (Pulmonata, Helicidae) from Sicily (Italy, Mediterranean Sea). Diversity in shell shape and size makes this group an ideal subject for quantitative studies of variability in land snails (Fig. 1). This genus includes a variable number of taxa (five species according to Manganelli et al., 1995; five species with 59 subspecies according to Bank, 2006). Unfortunately, due to punctiform distribution of shell morphs, loss of type material, generality of type locality (sometimes merely ‘Sicily’) and controversial application of names to different morphs, it is impossible to unequivocally assign each analysed population to a taxon at this stage of knowledge. The only exceptions are populations of *Marmorana muralis* having an easily recognized phenotype.

**MATERIAL AND METHODS**

**SAMPLING DESIGN AND BIOMETRIC MEASUREMENTS**

A two-factor design was used: area factor (6 km²) and site factor (100 m²). Two sites were randomly selected in each area; 31 areas and 57 sites (in five areas, two-site sampling was not possible for logistic reasons) were sampled (see Supplementary Material, Appendix S1). The sizes of areas and sites were defined arbitrarily on the basis of what little data are available with respect to the dispersion rate and population structure of pulmonates (de Winter & Gittenberger, 1998; Cameron, Mylonas & Vardinoyannis, 2000; Menez, 2001; Cameron et al., 2003). However, the size of areas matched the scale of the main geomorphic units in the region.

Sites within areas took into account possible variations in morphology at a scale smaller than area (Underwood, 1997). In this way, comparison between areas (regional spatial scale) was not biased by any high variability within areas (Underwood, 1997).

For each site, descriptive observations on exposure of rocks where specimens were sampled, altitude, and vegetation were made in the field. Data on mean annual temperature, evapotranspiration, annual precipitation, altitude, and climatic index were obtained from SIAS (2005).
Ten specimens were collected randomly in each site making 20 specimens per area and a total of 570 specimens. Only shells with a reflected lip were used because this attribute indicates cessation of growth and snail maturity. Live specimens were drowned in water, then fixed and preserved in 75% ethanol buffered with sodium carbonate (Manganelli et al., 2001, 2004).

Several shell features (LWW, last whorl width; HMaxD, hemi-maximum diameter; MinD, minimum diameter; MaxD, maximum diameter; AH, aperture height; AW, aperture width; SpH, spire height; ShH, shell height; MaxLWH, maximum last whorl height; MinLWH, minimum last whorl height; α, angle; β, angle) were measured to the nearest 0.01 mm using digital images (Adobe Photoshop, release 7.0.1). Specimens were photographed in standardized views (Fig. 2) using a Nikon Coolpix 4500 digital camera.

For each site five randomly selected, fully developed specimens, from those analysed for shell features, were dissected under a light microscope using fine pointed watchmaker’s tweezers. On the isolated genitalia, 12 linear variables were measured using a millimetric lens under a light microscope (0.01 mm): DV, length of distal vagina; PV, length of proximal vagina; DS, length of dart sac; MG, length of mucus glands; BCD1, length of proximal bursa copulatrix duct; BCD2, length of distal bursa copulatrix duct; BC, length of bursa copulatrix; DBC, length of bursa copulatrix diverticulum; EP, length of epiphallus; F, length of flagellum; PP, length of proximal penis; P, length of penis (Fig. 3). All the specimens studied are kept in the F. Giusti Collection (Dipartimento di Scienze Ambientali, Siena, Italy).

DATA ANALYSIS

Multivariate ordination by principal component analysis (PCA) was used to detect the main patterns of morphological variation in shells and genitalia. Three PCA were performed on the following matrix: 1, shell and genitalia; 2, genitalia; and 3, shell. The first matrix was analysed to detect general patterns of shell and genitalia variation and covariation whereas the latter two matrices were used for detailed analysis of the two data sets. In the three PCA, population coordinates were computed by intrapopulation averages used as supplementary rows. Before PCA, variables were log-transformed to obtain linear relationships. Size is generally the first determinant...
of biometric variation (Cadima & Jolliffe, 1996). Thus, techniques that separate size and shape components have recently been proposed and successfully employed in studies of shell biometry, also considering that snail shell size is usually not spatially structured, whereas shape can display geographical structure (Madec et al., 2003). Following Cadima & Jolliffe (1996), a shape-related PCA can be performed on a matrix $A$ (columns = log-transformed variables) by:

1. centring $A$ by columns into $X$;
2. obtaining $Z = XQ'$ where $Q' = I_p - (S_{a_0})(a_0'S_{a_0})^{-1}a_0'$; and
3. performing a covariance PCA of $Z$ (where $a_0'$ is the isometric size vector, $S$ is the covariance matrix of $A$ and $I_p$ is the identity matrix of order $p$, where $p$ is the number of variables). The method retains the property of uncorrelatedness of PCA components (Cadima & Jolliffe, 1996) and in this respect performs better than other PCA methods (such as double centring).

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**Figure 2.** Shell measurements. AH, aperture height; AW, aperture width; HMaxD, hemi-maximum diameter; LWW, last whorl width; MaxD, maximum diameter; MaxLWH, maximum last whorl height; MinD, minimum diameter; MinLWH, minimum last whorl height; ShH, shell height; SpH, spire height; $\alpha$, angle; $\beta$, angle.

**Figure 3.** *Marmorana* distal genitalia: outline and measurements. BC, bursa copulatrix; BCD1, proximal bursa copulatrix duct; BCD2, distal bursa copulatrix duct; DBC, bursa copulatrix diverticulum; DS, dart sac; DV, distal vagina; EP, epiphallus; F, flagellum; MG, mucus glands; P, penis; PP, proximal penis; PV, proximal vagina.
that separate size and shape components (Somers, 1989; Yoccoz, 1993).

Two-way analysis of variance (ANOVA; Bonferroni corrections for multiple comparisons) was used to detect significant differences between areas and within areas (sites) for each shell and genitalia variable and for the shape indices derived from shell (ShH/MaxD, LWW/ShH, SpH/MaxD, AH/AW). Areas with only one sampling site were excluded from this analysis.

To detect any relationship between shell biometry and environmental conditions (mean annual temperature, evapotranspiration, annual precipitation, altitude), redundancy analysis (RDA; Ter Braak, 1986) was used on the original matrix and the $Z$-matrix. Biometric variables were used to order populations and environmental variables were used as constraining explanatory factors (Legendre & Legendre, 1998). RDA was implemented by community ecologists to detect relationships between environmental data and species distribution but the method can virtually be used to describe relationships between any two data sets (Legendre & Legendre, 1998). A permutation test was used to test the significance of constraints on RDA for the first axis and the sum of all constrained eigenvalues (Oksanen et al., 2006).

All calculations were performed using R-package, version 2.3.0 (R Development Core Team, 2006). In particular, PCA and RDA analysis were performed with packages specifically designed for multivariate statistics: PCA was performed with the package ade4 (Chessel et al., 2005), and RDA using the package vegan (Oksanen et al., 2006).

All analyses (multi- as well as univariate) were also performed eliminating all populations belonging to the $M$. muralis phenotype, collected from ruderal habitats, from the data sets. This group of populations is readily distinguished, being widespread in Sicily only in this kind of habitat and subject to anthropochore dispersion (Sacchi, 1955). Because no differences were found between the two sets of analyses, only the results obtained for the data set including $M$. muralis populations are reported.

**RESULTS**

**MULTIVARIATE ANALYSIS**

PCA on shell and genitalia revealed size as the first major source of morphological variation (Table 1). The first axis explained 72% of the variance and inertia coefficients indicate that populations with high factor scores on PC1 are those of larger size. The PC2 (9%) inertia coefficients revealed a contrast between shell (negative coefficients) and genitalia (positive coefficients) variables. Populations with negative values on the second axis therefore had larger shells with respect to the genitalia.

PCA on genitalia explained 99% of the variance with PC1 only, which is a positive combination of all variables, therefore being a size component (Table 2). PCA on shell confirmed size as the first determinant of variance because PC1 (56%) is a positive combination of all variables. By contrast, PC2 (14%) accounted for a shape component being a contrast between variables related to shell height and those related to shell width. This shape component describes shell globosity, traditionally measured with

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1 (0.72)</th>
<th>PC2 (0.09)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH</td>
<td>7010</td>
<td>-1347</td>
</tr>
<tr>
<td>AW</td>
<td>5998</td>
<td>-2836</td>
</tr>
<tr>
<td>MaxD</td>
<td>6117</td>
<td>-3072</td>
</tr>
<tr>
<td>MinD</td>
<td>5681</td>
<td>-2615</td>
</tr>
<tr>
<td>HMaxD</td>
<td>5463</td>
<td>-1732</td>
</tr>
<tr>
<td>LWW</td>
<td>912</td>
<td>-1116</td>
</tr>
<tr>
<td>ShH</td>
<td>7968</td>
<td>-235</td>
</tr>
<tr>
<td>SpH</td>
<td>4694</td>
<td>111</td>
</tr>
<tr>
<td>MinLWH</td>
<td>8107</td>
<td>-715</td>
</tr>
<tr>
<td>MaxLWH</td>
<td>8062</td>
<td>-782</td>
</tr>
<tr>
<td>α</td>
<td>64</td>
<td>-182</td>
</tr>
<tr>
<td>β</td>
<td>-1178</td>
<td>-647</td>
</tr>
<tr>
<td>P</td>
<td>9493</td>
<td>310</td>
</tr>
<tr>
<td>PP</td>
<td>9474</td>
<td>377</td>
</tr>
<tr>
<td>EP</td>
<td>9450</td>
<td>368</td>
</tr>
<tr>
<td>F</td>
<td>9480</td>
<td>378</td>
</tr>
<tr>
<td>DV</td>
<td>9363</td>
<td>493</td>
</tr>
<tr>
<td>DS</td>
<td>9364</td>
<td>512</td>
</tr>
<tr>
<td>MG1</td>
<td>9331</td>
<td>531</td>
</tr>
<tr>
<td>MG2</td>
<td>9355</td>
<td>517</td>
</tr>
<tr>
<td>PV</td>
<td>9375</td>
<td>458</td>
</tr>
<tr>
<td>BCD1</td>
<td>9452</td>
<td>446</td>
</tr>
<tr>
<td>BCD2</td>
<td>9382</td>
<td>460</td>
</tr>
<tr>
<td>DBC</td>
<td>9443</td>
<td>398</td>
</tr>
</tbody>
</table>

Total inertia of each PC is indicated in parenthesis.

AH, aperture height; AW, aperture width; MaxD, maximum diameter; MinD, minimum diameter; HMaxD, hemi-maximum diameter; LWW, last whorl width; ShH, shell height; SpH, spire height; MinLWH, minimum last whorl height; MaxLWH, maximum last whorl height; α, angle; β, angle; P, length of penis; PP, length of proximal penis; EP, length of epiphallus; F, length of flagellum; DV, length of distal vagina; DS, length of dart sac; MG, length of mucus glands; PV, length of proximal vagina; BCD1, length of proximal bursa copulatrix duct; BCD2, length of distal bursa copulatrix duct; DBC, length of bursa copulatrix diverticulum.

simple indices such as the ratio of shell height to width. Shape-related PCA based on the method of Cadima & Jolliffe (1996) therefore indicates that ShH and MaxD are the two principal shape determinants (Figs 4, 5). Indeed, these two variables have the largest relative contributions to the total inertia of PC1 (Table 3).

On PC2 (24%), β, which is related to aperture inclination, contrasted with the MinLWH and MaxLWH (Table 3). PC2 therefore distinguishes populations with higher aperture inclination and relatively smaller last whorl and populations with the opposite relationship.

RDA on size-constrained (A) and shape-related shell (Z) matrices can not significantly constrain (Permutation = 999: pseudo-F for all eigenvalues = 1.09, P > 0.05; pseudo-F for the first eigenvalue = 2.86) environmental variables on the multivariate axis obtained by reciprocal ordering of populations and shell variables (Fig. 6).

Thus, area shell–shape components are not significantly influenced by environmental factors (climatic or geomorphological). Different shell shapes occupied areas with similar climatic parameters. In some cases, field observations also showed that the same shell shapes occurred in apparently different habitats (e.g. ruderal habitats, natural habitats, and habitats with different vegetation cover).

Table 2. Relative contribution of each variable to the inertia of the first two principal component analysis components (genitalia data set)

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1 (0.99)</th>
<th>PC2 (&lt; 0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>9878</td>
<td>–88</td>
</tr>
<tr>
<td>PP</td>
<td>9916</td>
<td>–64</td>
</tr>
<tr>
<td>EP</td>
<td>9919</td>
<td>–64</td>
</tr>
<tr>
<td>F</td>
<td>9948</td>
<td>–43</td>
</tr>
<tr>
<td>DV</td>
<td>9942</td>
<td>0</td>
</tr>
<tr>
<td>DS</td>
<td>9955</td>
<td>–1</td>
</tr>
<tr>
<td>MG1</td>
<td>9945</td>
<td>–1</td>
</tr>
<tr>
<td>MG2</td>
<td>9943</td>
<td>31</td>
</tr>
<tr>
<td>PV</td>
<td>9897</td>
<td>65</td>
</tr>
<tr>
<td>BCD1</td>
<td>9938</td>
<td>44</td>
</tr>
<tr>
<td>BCD2</td>
<td>9920</td>
<td>56</td>
</tr>
<tr>
<td>DBC</td>
<td>9910</td>
<td>40</td>
</tr>
</tbody>
</table>

Total inertia of each PC is indicated in parenthesis.
P, length of penis; PP, length of proximal penis; EP, length of epiphallus; F, length of flagellum; DV, length of distal vagina; DS, length of dart sac; MG, length of mucus glands; PV, length of proximal vagina; BCD1, length of proximal bursa copulatrix duct; BCD2, length of distal bursa copulatrix duct; DBC, length of bursa copulatrix diverticulum.

Table 3. Relative contribution of each variable to the inertia of the first two PCA components (shell data set, Z-matrix for shape-related PCA)

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1 (0.31)</th>
<th>PC2 (0.24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH</td>
<td>1131</td>
<td>–1566</td>
</tr>
<tr>
<td>AW</td>
<td>3471</td>
<td>–322</td>
</tr>
<tr>
<td>MaxD</td>
<td>8370</td>
<td>–261</td>
</tr>
<tr>
<td>MinD</td>
<td>4679</td>
<td>–622</td>
</tr>
<tr>
<td>HmaxD</td>
<td>3118</td>
<td>–2497</td>
</tr>
<tr>
<td>LWW</td>
<td>1870</td>
<td>139</td>
</tr>
<tr>
<td>ShH</td>
<td>–8413</td>
<td>–867</td>
</tr>
<tr>
<td>SpH</td>
<td>–7177</td>
<td>678</td>
</tr>
<tr>
<td>MinLWH</td>
<td>–2065</td>
<td>–6582</td>
</tr>
<tr>
<td>MaxLWH</td>
<td>–2785</td>
<td>–5586</td>
</tr>
<tr>
<td>α</td>
<td>1375</td>
<td>4354</td>
</tr>
<tr>
<td>β</td>
<td>717</td>
<td>6591</td>
</tr>
</tbody>
</table>

Total inertia of each PC is indicated in parenthesis.
AH, aperture height; AW, aperture width; MaxD, maximum diameter; MinD, minimum diameter; HMaxD, hemi-maximum diameter; LWW, last whorl width; ShH, shell height; SpH, spire height; α, angle; β, angle.
Figure 5. Shape-related principal component analysis ordination plot (PC1 versus PC2) of areas. Population coordinates were obtained by computing intrapopulation averages as supplementary rows. For acronyms, see Supplementary Material, Appendix S1.

Figure 6. Redundancy analysis (RDA) constraint ordination of shape-related shell variables and environmental variables. Arrow direction and length show degree of correlation between environmental variables and RDA axis. AH, aperture height; Alt, altitude; AW, aperture width; Evap, evapotranspiration; HMaxD, hemi-maximum diameter; LWW, last whorl width; MaxD, maximum diameter; MaxLWH, maximum last whorl height; MinD, minimum diameter; MinLWH, minimum last whorl height; Prec, annual precipitation; ShH, shell height; SpH, spire height; T, mean annual temperature; α, angle; β, angle.
water stress conditions and, when associated with a
character varies as an adaptation to local environ-
mental conditions (i.e. selection), other characters
may vary due to the developmental constraint,
although not adaptively (Gould, 1984).

ANOVA

Regarding shell variables, ANOVA (Table 4) detected
significant differences for areas and sites. By contrast,
shell indices only showed significant differences for
area.

Regarding genitalia variables, half of the 12 vari-
ables showed significant variation for area only and
the other six variables had significant variation for
areas and sites (Table 5).

DISCUSSION

SHELL VARIABILITY: AREA SCALE

Patterns of shell shape and size are usually analysed
on a regional scale to reveal congruencies between
morphological and geographical patterns and to iden-
tify neutral versus selective processes (Goodfriend,
1986; Kempermann & Gittenberger, 1988; Johnson &
Black, 2000; Welter-Schultes, 2000; Pfenninger &
Magnin, 2001). Regarding shell adaptation to the
environment, several hypotheses have been proposed
(Goodfriend, 1986; Johnson & Black, 2000; Welter-
Schultes, 2000). Concerning shape, a small aperture
was thought to reduce the exposed surface under
water stress conditions and, when associated with a

Table 4. Two-way analysis of variance (site randomly
nested in area)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area</th>
<th>Site</th>
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<tr>
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<td>F_{25,26}</td>
<td>P</td>
</tr>
<tr>
<td>AW</td>
<td>29.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MaxD</td>
<td>57.53</td>
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<tr>
<td>MinD</td>
<td>69.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HMaxD</td>
<td>30.12</td>
<td>&lt; 0.001</td>
</tr>
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<td>LWW</td>
<td>43.73</td>
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</tr>
<tr>
<td>ShH</td>
<td>19.51</td>
<td>&lt; 0.001</td>
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<tr>
<td>MinLWH</td>
<td>136.71</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MaxLWH</td>
<td>46.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>α</td>
<td>130.96</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>β</td>
<td>148.21</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ShH/MaxD</td>
<td>3.74</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LWW/ShH</td>
<td>6.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SpH</td>
<td>96.78</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MinLWH</td>
<td>36.64</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MaxLWH</td>
<td>35.92</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AH/AW</td>
<td>10.39</td>
<td>&lt; 0.001</td>
</tr>
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</table>

AH, aperture height; AW, aperture width; MaxD,
maximum diameter; MinD, minimum diameter; HMaxD,
hemi-maximum diameter; LWW, last whorl width; ShH,
shell height; SpH, spire height; MinLWH, minimum last
whorl height; MaxLWH, maximum last whorl height; α, angle; β, angle.

Table 5. Two-way analysis of variance (site randomly
nested in area)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>18.10</td>
<td>&lt; 0.001</td>
</tr>
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<td>PP</td>
<td>8.32</td>
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<td>F</td>
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<td>&lt; 0.001</td>
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<tr>
<td>DS</td>
<td>15.72</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MG1</td>
<td>19.47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MG2</td>
<td>9.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PV</td>
<td>12.46</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BCD1</td>
<td>6.10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BCD2</td>
<td>18.80</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBC</td>
<td>30.60</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

P, length of penis; PP, length of proximal penis; EP, length
of epiphallus; F, length of flagellum; DV, length of distal
vagina; DS, length of dart sac; MG, length of mucus
glands; PV, length of proximal vagina; BCD1, length of
proximal bursa copulatrix duct; BCD2, length of distal
bursa copulatrix duct; DBC, length of bursa copulatrix
diverticulum.

flat shell, to allow deeper penetration into the veg-
etation or under stones (Goodfriend, 1986). Although
several shell traits are correlated with spatial vari-
ations in environmental parameters, Goodacre (2001)
demonstrated that genetic clines occur at regional
scale in relation to significant morphological changes
(but without significant environmental changes) and
claimed that there is a neutral component to shell
shape and size, as well as selection.

The conclusion that shell shape in Sicilian Marmo-
rana is not significantly influenced by environmental
factors is in line with some other case studies
analysing shape on the shell height/shell diameter
ratio (intertidal gastropods: Johnson & Black, 2000;
land snails: Goodfriend, 1986; Welter-Schultes, 2000;
Madec et al., 2003) and with an analysis of variations
in spatial distribution of Cornu aspersum (Madec et al.,
2003).

Because there is no clear evidence of environmental
constraint, a first hypothesis is that area scale vari-
ability could be generated by past factors such as
neutral or selective processes that have ceased to act.
Alternatively, a second hypothesis is that shell vari-
ability could be generated by developmental corre-
lates. Developmental correlates indicate that, when a
caracter varies as an adaptation to local environ-
mental conditions (i.e. selection), other characters
may vary due to the developmental constraint,
although not adaptively (Gould, 1984).
Although our results do not allow us to definitely discriminate either hypothesis, they establish some interesting points. Shape-related PCA indicated a negative relationship between spire/shell height and last whorl/hemi-maximum diameter length and diameter, as well as a negative relationship between inclination of aperture (β) and last whorl height. Consequently, flat or depressed shells with a wider last whorl and taller spire have less tilted apertures. Deflection of aperture has been interpreted as an adaptation to reduce water loss in resting specimens (Mazek-Fialla, 1934), or to lower the centre of gravity to reduce dislodgement during inactivity (McNair et al., 1981). As for the former hypothesis, RDA analysis showed inclination of aperture (β) to be weakly correlated with temperature and evapotranspiration. As for the second one, PCA results were in line with the expectation that aperture deflection acts as balancer to reduce dislodgement with increasing spire height, which is a matter of special importance for rock-dwelling species.

**Shell Variability: Site Scale**

The main result of multiple scale analysis was a significant morphological variation at regional (differences between areas) and local (differences between sites) scales indicated by various biometric parameters. Although areas, or groups of areas, were significantly different, sites within areas were sometimes also significantly different. Notably, ANOVA showed that such site variability was mainly due to shell size. All shell linear variables were significant for areas as for sites, although shell indices, which describe shape, changed significantly among areas only. The two-scale analysis of *Marmorana* shell morphometry therefore suggests that populations are separated from each other by morphometric gradients of variability over a local scale where the habitat appears to be uniform (a morphological ‘area effect’ at local scale).

Site shell size variability has been explained by direct environmental influences such as moisture, temperature, calcium carbonate substrate content (although certain European and Mediterranean species failed to reveal any relationship), population density, and, recently, pollution (Goodfriend, 1986; Boulding & Hay, 1993; Pfenninger & Magnin, 2001) or indirect influences (for a review, see Goodfriend, 1986). Other studies (Gould & Woodruff, 1978, 1990; Welter-Schultes, 2000), however, failed to find any clear correlation with environmental factors. Moreover, breeding experiments have shown that a large part of morphotype differentiation is genetically determined but, within a given morph, variation may be induced by environmental components (Madec & Guiller, 1993; Madec et al., 1998). Madec et al. (2003), who first effectively distinguished between shape and size variability and their spatial variation, illustrated how shell size failed to show any such geographical cleavage and how a high degree of interpopulation variation was largely environmentally determined. Interpopulation shell sizes belonging to the same shape are often influenced by local conditions and show a high degree of phenotypic plasticity, frequently being involved in life-history tactics (Madec & Daguzan, 1993; Madec, Desbuquois & Coutellec-Vreto, 2000).

Despite this evidence of shell size variability, sharp shell size differentiation in restricted areas has previously been the basis for assigning ‘varietal’ names to *Marmorana* and many other helicid snails (i.e. *C. aspersum*; Madec et al., 2003).

**Anatomical Variability: Area and Site Scales**

Little is known about anatomical variability and its correlation with shell variability. Our results show an absence of sharp changes in genitalia measurements, unlike for shell. However, certain populations show allometries between shell size and genitalia measurements without any apparent geographical pattern. Madec et al. (2003) found such allometric relationships between shell size and genitalia measurements, albeit between populations of *C. aspersum* distributed in two different geographical areas (western and eastern populations of North Africa) and they postulated mechanical constraints, possibly involving precopulatory isolation in a contact zone. Previous studies of variation of distal genitalia of *C. aspersum* demonstrated agreement with spatial pattern (Madec & Guiller, 1994; Madec et al., 2003). Madec et al. (2003) also showed a progressive decrease in morphometric similarity among populations within a geographical distance range.

As shown by ANOVA, certain genitalia measurements varied at the area scale only, whereas others varied at both area and site scales. Thus, on average, significant changes in shell size between sites does not necessarily correspond to significant changes in genitalia measurements.

The multiple scale variation patterns observed in *Marmorana* are consistent with the scenarios proposed in other studies. In some species, genital variance may be selectively controlled to maintain function (Baminger & Haase, 2000), such as the strong control of the number of allo sperms (Lind, 1973; Haase & Baur, 1995) or differences in the number of sperm transferred (Locher & Baur, 1997; Baur, Locher & Baur, 1998). Although little data have yet been collected on genital evolution in *Marmorana* (and it is generally beyond the scope of the present
study), such a pattern (allometry as well as selective control) may have important mechanical implications that could lead to precopulatory isolation between populations with allopatric or parapatric distribution.

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**SUPPLEMENTARY MATERIAL**

The following material is available for this article online:

**Appendix S1.** List of the areas and sites, altitudes, Universal Transverse Mercator geographic coordinates, collectors, dates, and accession numbers.

This material is available as part of the online article from:


(This link will take you to the article abstract).

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