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SHELL SHAPE VARIATION IN *LITTORINA SAXATILIS* AND *L. ARCANA*: A CASE OF CHARACTER DISPLACEMENT?

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(Figures 1-7)

Variation in shape of the shell of *Littorina saxatilis* and *Littorina arcana* has been investigated using shells from four shores where the species co-occur and seven where the former only is present. Samples were taken from shores along the south-west and south coasts of Britain, between Cardigan Bay and Kent. Measurements of eight shell variables and of the operculum area were analysed using canonical variate analysis. The results of the analysis suggest that the shells can be separated into three groups, namely *L. arcana, L. saxatilis* where it is sympatric with *L. arcana*, and *L. saxatilis* where it is allopatric from *L. arcana*. The separation of the two groups of *L. saxatilis* is based primarily on their relative globosity, whereas the separation of *L. saxatilis* from *L. arcana* is based primarily on the relative size of the operculum and width of shell whorl one (the first above the body whorl). Sexual dimorphism has been considered and shown not to contribute significantly to the between-shore pattern of variation observed. The relationships between important variables were further investigated using ANCOVA, and the relationship of foot area to the shell variables was also analysed at four of the shores. It is concluded that there is evidence of character displacement in *L. saxatilis*, shown by an increase in globosity in the presence of *L. arcana*, and that this may partly explain the continuing uncertainty over the taxonomic and ecological identity of members of the rough periwinkle group.

INTRODUCTION

It is now well established that there are several species in the group of rough periwinkles inhabiting European rocky shores. The usual consensus agrees on four, namely *Littorina saxatilis* (Olivi), *Littorina arcana* Ellis, *Littorina nigrolineata* Gray and *Littorina neglecta* Bean. The first two species are particularly difficult to distinguish in the field and have received much attention. Recent work indicates that the ovoviviparous *L. saxatilis* is a very variable species living in a wide range of habitats (*e.g.* Janson, 1985) and that, although it is close to the oviparous *L. arcana* in shell morphology and in genotype, the two are separate species (Ward & Warwick, 1980; Ward & Janson, 1985), and not reproductive morphs of the same species.

Janson & Sundberg (1983) described three morphs within a few metres of one another on the shore of Salto island, Sweden. The 'E' morph had a relatively large aperture and thin shell, the 'S' morph had a smaller aperture and thicker shell. They occurred on exposed and sheltered portions of the shore, respectively, and were separated by less than 5 m. Shells of intermediate ('I') form were found in this intervening zone. Heller (1976), Raffaelli (1979) and Atkinson & Newbury (1984) have all

similarly described *L. saxatilis* (=*L. rudis*) from exposed shores with larger apertures and also foot areas (Atkinson & Newbury, 1984) than possessed by those from sheltered shores. Grahame & Mill (1986) showed that in both *L. saxatilis* and *L. arcana* aperture size and foot area increased with exposure on a shore in south Wales. However they noted that at Portland Bill on the south coast of England *L. saxatilis* achieved a larger foot area than it was apparently able to do on the Welsh shore, and after examination of specimens from the British Museum (Natural History) it was apparent that a broader scale of variation on exposed shores might exist.

Daguzan (1977) has shown that in a population of *L. saxatilis* on a rocky point at Penvins (Morbihan, Brittany), a measure of the aperture width was relatively smaller in mature females than in mature males. Fretter & Graham (1962) stated that females had a relatively round aperture compared with males. In both these cases there is the possibility of confounding of *L. arcana* in the original samples, since the latter species was unknown to these authors. Van Marion (1981) found that *L. saxatilis* showed no sexual dimorphism on a sheltered boulder beach at Trondheim, Norway, where it is almost certainly the only species of rough periwinkle on the shore.

In this paper we examine the status of populations of *L. arcana* and of large ovoviviparous winkles (thus excluding the small *L. neglecta*, which is also ovoviviparous) from several shores in southern Britain. Smith (1981) suggested that '*L. saxatilis*' may comprise two species, *L. saxatilis* sensu stricto (with a comparatively large aperture) and *Littorina rudis* (Maton) with a comparatively small aperture. On the basis of the results described below and in a study of the esterase enzyme system (Mill & Grahame, 1988), the large ovoviviparous species will be referred to as *L. saxatilis* irrespective of the size of its aperture.

METHODS

Collections of *Littorina* were made on shores along the southwest and south coasts of Britain, between Cardigan Bay and Kent (Figure 1). All the sites were exposed to the open sea, and were chosen so as to be as similar as possible to one another in showing maximum exposure for that portion of the coast. Bodies were extracted from the shells after boiling, or in some cases after deep freezing and thawing, and determined for sex and species. The only wholly reliable criterion for distinguishing *L. saxatilis* from *L. arcana* is among females, where the presence of a brood pouch indicates *L. saxatilis* and a jelly gland indicates *L. arcana*.

It was found that *L. saxatilis* and *L. arcana* were present together at Great Castle Head (Westdale Bay), Dyfed (SM/797056); Duckpool (SS/199114), and Trevaunance (SW/725519), Cornwall; and at Prawle Point, south Devon (SX/775351). *L. saxatilis* only was found at Cae-du (SH/565059), Gwynedd; Golden Cap (SY/407918), Portland Bill (SY/675683) and St Alban's Head (SY/959754), Dorset; Alum Bay (SZ/304851) and St Catherine's Point (SZ/496753), Isle of Wight; and St Margaret's at Cliffe (TR/368444), Kent. At Duckpool, Trevaunance and Prawle Point, where the two species occur together, only females have been used. On the remaining shore of co-occurrence, Great Castle Head, after experience using the female reproductive tract character as an initial
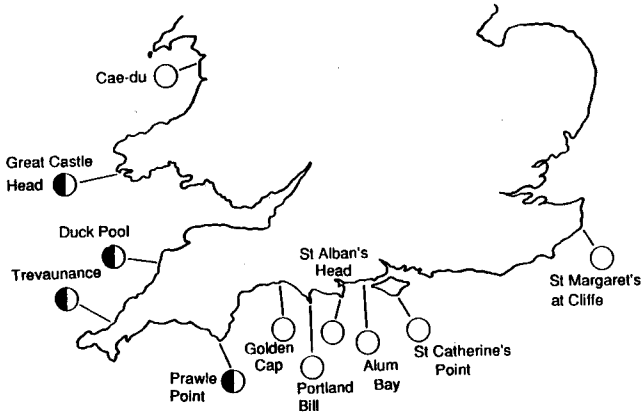
Figure 1. Map of southern Britain showing location of sites. Open circles, L. *saxatilis* present alone (allopatric); half solid circles, L. *saxatilis* and L. *arcana* present together (sympatric).

criterion, the appearance of the shells was found to be sufficiently distinct to separate the species, and hence males from this shore have been included in the analysis. Throughout, great care was taken to use only material which was attributable unambiguously to one species or the other, relying on female body characters for discrimination.Sample sizes are:

Cae-du, *L. saxatilis*, 27(F);
Great Castle Head, *L. saxatilis*, 29(F), 4(M); *L. arcana*, 37(F), 22(M);
Duckpool, *L. saxatilis*, 21(F);
Trevaunance cliff, *L. saxatilis*, 26(F), *L. arcana*, 15(F);
Trevaunance boulders, *L. saxatilis*, 7(F);
Prawle Point, *L. saxatilis*, 29(F); *L. arcana*, 28(F);
Golden Cap, *L. saxatilis*, 19(F), 11(M);
Portland Bill, *L. saxatilis*, 11(F), 8(M);
St Alban's Head, *L. saxatilis*, 11(F), 10(M);
Alum Bay, *L. saxatilis*, 6(F), 7(M);
St Catherine's Point, *L. saxatilis*, 31(F);
St Margaret's at Cliffe, *L. saxatilis*, 17(F), 11(M).

Measurements were taken using a digitizer tablet and microcomputer. The shell variables measured for this study are shown in Figure 2. The shell and operculum were placed on the stage of a dissecting microscope with a drawing tube, and the measurements were digitized directly from the image on the tablet, with the exception of the apical angle which was derived from an algorithm. The variables were selected so as to obtain a set of numerical values summarising the shell attributes of length, width and globosity, and also operculum area. This was used instead of aperture area, as it was judged to be more difficult reliably to define the latter measurement repeatedly on different shells. In addition, for animals from some of the shores, foot areas were measured as described by Grahame & Mill (1986).

Examination of the values for the linear and area measurements showed that, al-

though in most samples they were normally distributed as untransformed data, there were several departures from normality. These variables were thus normalised by transformation to logarithms to base 10 before analysis. The values for apical angle were all normally distributed; these were not transformed before analysis.
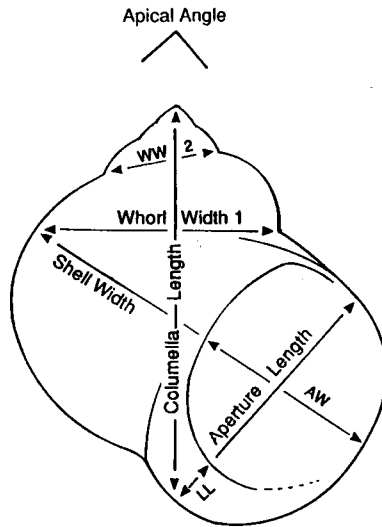

Figure 2. Shell of *Littorina saxatilis* showing the eight variables measured. aw, aperture width; ll, lip length; ww2, whorl width two.

The measurements were first analysed using canonical variate analysis (Campbell & Atchley, 1981; Reyment *et al.*, 1984) in the Statistical Analysis System (SAS) package (SAS Institute Inc., 1985). In this analysis the procedure CANDISC was used to find those linear combinations of variables (discriminant functions) which best reveal differences between the populations. Thus a series of discriminant functions is calculated, consisting of coefficients each of which represents a weighting giving the relative contribution of the original variables to separation of the populations. This is represented in the expression

$$z = b_1 x_1 + b_2 x_2 \ldots + b_n x_n$$

where z is a discriminant function and the successive b's form a vector of coefficients for weighting the variables (x's). In canonical variate analysis the successive sets (vectors) of b values for each of the discriminant functions (z's) are said to represent underlying axes of variation, the canonical variates, which are chosen (by the program) to be uncorrelated with one another. For calculation of the F statistics associated with the Mahalanobis distance, the procedure DISCRIMINANT in the SPSS™ package was used (SPSS, 1986) as it was found that CANDISC in SAS performs this test wrongly when group sizes are unequal.

Further investigation of important variables identified in the canonical variate analysis was carried out using analysis of covariance and stepwise multiple regression.

RESULTS

At Duckpool there were no *L. arcana* in the sample for measurement, so although it does occur there this species is not represented in the data for that shore. At Trevaunance we have two samples from contrasting locations. Both species inhabit the same crevices on a vertical rock cliff, but in the boulders formed from the disintegration of a jetty *L. saxatilis* is found without *L. arcana*. We nevertheless regard this latter as a 'sympatric' situation as *L. arcana* is found within a very few metres of the boulders.

Canonical variate analysis

The distribution of these species in Britain is being investigated by us and will be reported separately (Mill & Grahame, 1989); for the present it should be noted that *L. saxatilis* seems to be the more widespread species and occurred on all the shores in the present study, while *L. arcana* did not occur in northeast Cardigan Bay or east of Lyme Regis on the Channel coast, and hence was found on only four of the shores reported on here - namely, Great Castle Head, Duckpool, Trevaunance and Prawle Point. Therefore these four are shores where *L. saxatilis* is sympatric with *L. arcana*; on the others *L. saxatilis* is allopatric from *L. arcana*.
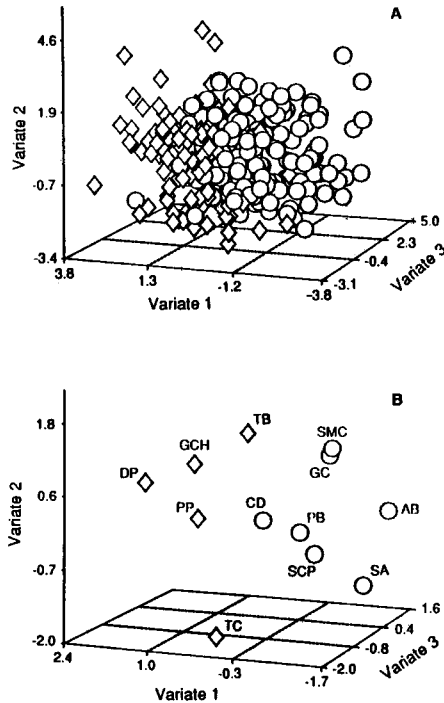
Figure 3. Canonical variate scores of (A) the individual shells and (B) the site means of *L. saxatilis* shown plotted on the first three canonical variates. A three-dimensional plot has been used because in the case of the individual shells this is much clearer. The sites are, from north-west to south-east: **Sympatric** - GCH, Great Castle Head; DP, Duckpool; TB, Trevaunance (boulders); TC, Trevaunance (cliff); PP, Prawle Point; **Allopatric** - CD, Cae-du; GC, Golden Cap; PB, Portland Bill; SA, St Alban's Head; AB, Alum Bay; SCP, St Catherine's Point; SMC, St Margaret's at Cliffe. ○, allopatric from *L. arcana*; ◇, sympatric with *L. arcana*.

In the first analysis the twelve *L. saxatilis* samples were considered. Nine canonical variates were calculated, each associated with a canonical correlation coefficient. The first eight correlation coefficients were significant (likelihood ratio test, P=0·0001 for correlation coefficients one to six, P=0·0002 for correlation coefficient seven, P=0·0108 for correlation coefficient eight and P=0·88 for correlation coefficient nine). For these first eight correlation coefficients, the proportions of variance associated with them (estimated from the sizes of the eigenvalues calculated from the canonical correlation coefficients) were 33% (1), 26% (2), 14% (3), 11% (4), 7% (5), 4% (6), 2·2% (7) and 1·9% (8), giving a total of 99·1%. It is usual for the first few canonical variates to represent the important inter-population variation (Reyment *et al.*, 1984), and inspection of the results showed that beyond variate two there was no evident meaning in the distribution of individual shells when plotted on the variates. Interpretation has accordingly been restricted to the first two canonical variates.

Figure 3A shows the scatter diagrams obtained by plotting the positions of the individual shells on the first three canonical variates for each of the twelve samples of *L. saxatilis*. In Figure 3B the scatter diagram of the sample means on the variates is given.

If the samples represented in Figure 3 are divided into two groups, namely those *L. saxatilis* sympatric with *L. arcana* and those allopatric from it, it is clear that the sympatric samples (at Great Castle Head, Duckpool, Trevaunance and Prawle Point) are furthest to the left on canonical variate one. It is noteworthy that the means for the two Trevaunance samples, while being at extreme ends of variate two, are similarly placed on variate one. Figure 4 shows for each sample the shell lying closest to the mean point with respect to the first 3 canonical variates (*i.e.* the 'centroid shells').

There are apparent differences between the samples; these were tested using the Mahalanobis distance and its associated F statistic (Reyment *et al.*, 1984). In this application the degrees of freedom for F are the number of variables measured (nine) and the quantity (observations - groups - variables + 1). With 285 shells from twelve samples (groups) and nine variables, the latter is 265. As there are twelve samples, there are 66 comparisons, which causes difficulties in the interpretation of the significance of multiple tests. A conservative solution to this problem is to divide the desired level of probability by the number of tests (comparisons) to obtain a new level of probability against which the computed values of F may be tested. Thus the 5% level should be tested using the probability level P=0·0008 (*i.e.* 0·05/66). Using this criterion, samples from two pairs of shores were found not to be significantly different, namely Golden Cap and St Margaret's at Cliffe, F=2·620 (P=0·0065), and Portland Bill and St Catherine's Point, F=2·125 (P=0·0278). The other samples were all significantly different from one another.

We have repeated the analysis on *L. saxatilis* keeping separate the data from those shores where we have included males in the samples for analysis. The sample means are shown plotted on the first three canonical variates in Figure 5. In each case the sexes are shown as being different, although the general pattern of Figure 3 is not disturbed. Only at Alum Bay (P=0·0768) and Golden Cap (P=0·0267) is there significance at near the P=0·05 level. Adopting the more rigorous criterion used above of P=(0·05/6) since there are 6 comparisons, none of the samples would be significantly different at this
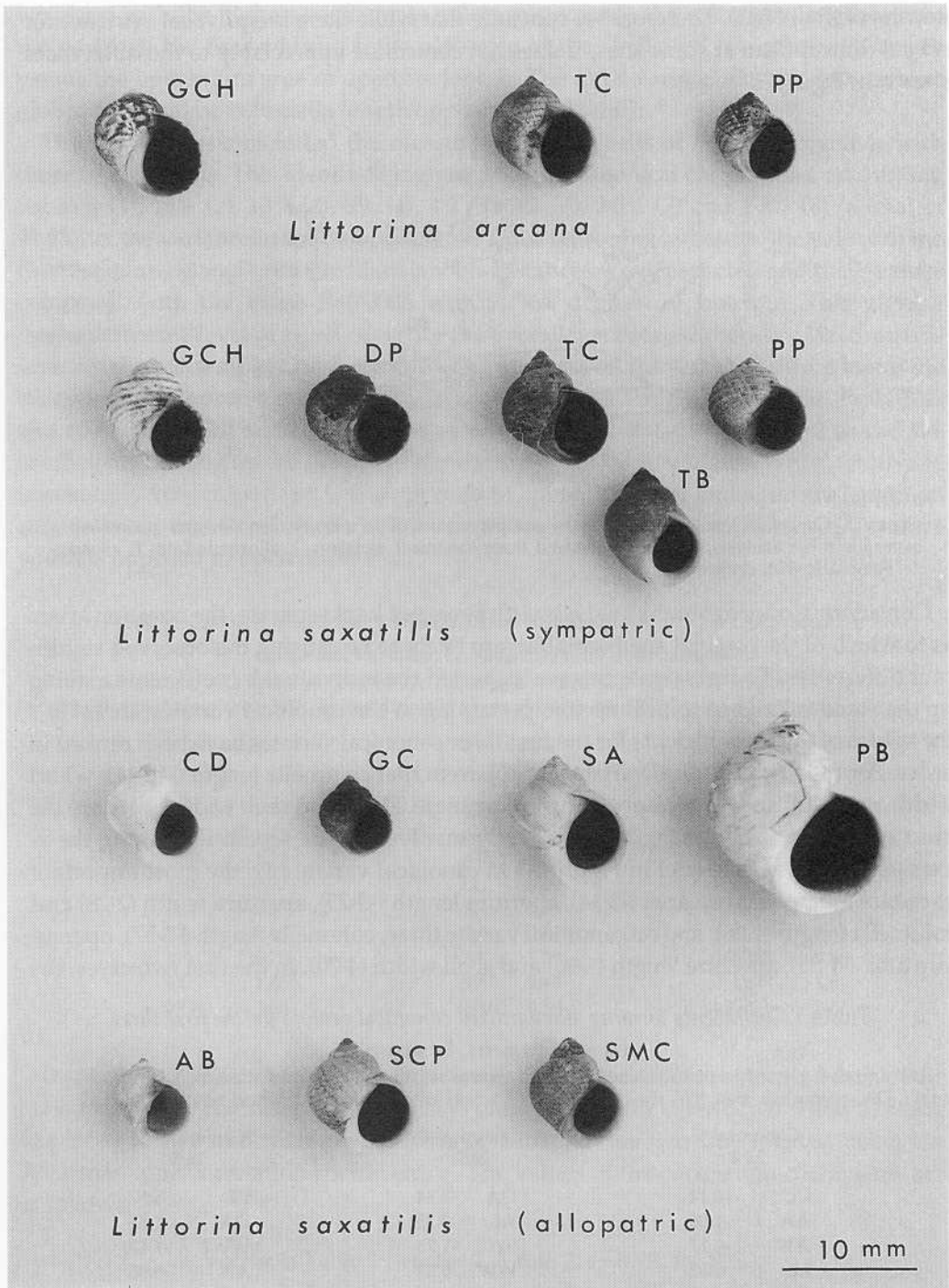
Figure 4. Shells from the samples measured. The shells chosen are those lying closest to the mean point for each sample with respect to the first three canonical variates (*i.e.* the 'centroid shells'). The sites are, from north-west to south-east: **Sympatric** - GCH, Great Castle Head; DP, Duckpool; TB, Trevaunance (boulders); TC, Trevaunance (cliff); PP, Prawle Point; **Allopatric** - CD, Cae-du; GC, Golden Cap; PB, Portland Bill; SA, St Alban's Head; AB, Alum Bay; SCP, St Catherine's Point; SMC, St Margaret's at Cliffe. The relatively globose shape of the shells of *Littorina saxatilis* at Great Castle Head, Duckpool and Prawle Point (sympatric with *L. arcana*) is obvious. Those from Trevaunance (also sympatric) are not so globose; Figure 3B shows these shells to lie near the allopatric samples.

new level (P=0·0083). Therefore we conclude that while there is equivocal evidence for sexual dimorphism at some sites, it does not contribute appreciably to the differences shown in Figure 3.
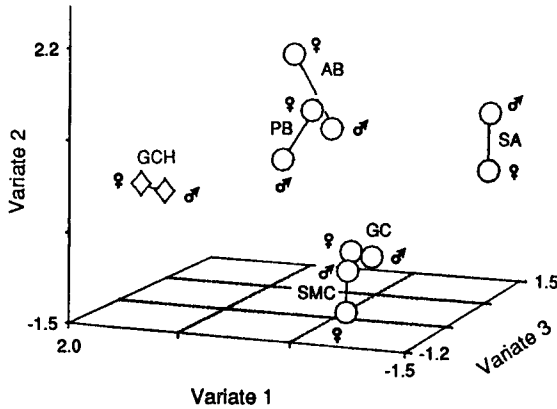
Figure 5. Canonical variate scores of the sample means of *L. saxatilis*, females and males kept separate in the analysis, plotted on the first three canonical variates. ○, allopatric from *L. arcana*; ◇, sympatric with *L. arcana*.

Considering once again the analysis with sexes not kept separate, the question arises as to which of the original shell variables are likely to be causing the observed significant differences. To investigate this we inspected the individual b coefficients making up the standardized canonical vectors pertaining to the canonical variates. In Table 1 the values of these coefficients for the first three canonical variates have been ranked in order. For the first canonical variate it is apparent that columella length (-10·14), whorl width one (5·13) and, to a lesser extent, apical angle (-1·00) and shell width (2·19) are the most important variables, and are evidently involved in the separations along the x-axes (canonical variate one) in Figure 3. On canonical variate two the most important variables are operculum area (-3·34), aperture length (-3·25), aperture width (2·28) and columella length (4·06); and on canonical variate three, columella length (-5·57), operculum area (-1·85), aperture length (1·96) and shell width (4·70). In the first two cases, the

Table 1.*Coefficients forming standardized canonical vectors for the first three canonical variates*, L. saxatilis

AA, apical angle; AL, aperture length; AW, aperture width; CL, columella length; LL, lip length; OA, operculum area; SW, shell width; WW1, whorl width one; WW2, whorl width two.

Canonical 1 33%		Canonical 2 26%		Canonical 3 14%	
CL	-10.14	OA	-3.34	CL	-5.57
AA	-1.00	AL	-3.25	OA	-1.85
AW	-0.17	SW	-1.75	WW2	-1.47
LL	0.05	WW1	0.24	AA	-0.95
OA	0.18	LL	0.49	WW1	-0.59
AL	1.08	AA	1.56	LL	0.15
WW2	1.21	WW2	1.57	AW	1.88
SW	2.19	AW	2.28	AL	1.96
WW1	5.13	CL	4.06	SW	4.70

variables with the highest coefficients reflect respectively the columella length versus the width of the first whorl, *i.e.* the globosity of the shell, and the columella length versus the operculum area or aperture length. The third variate, like the first, reflects globosity, this time columella length opposing shell width.

The next analysis included the measurements on shells of *L. arcana* together with those of *L. saxatilis*. This identified eight significant canonical correlations, accounting for 46% (1), 22% (2), 13% (3), 8% (4), 4% (5), 3% (6), 2·6% (7) and 1·3% (8) (a total of 99·9%) of the variance in the data. Using the same reasoning as before, the values of the F statistics associated with the Mahalanobis distances were inspected and the P values compared with the value P=0·0005 with 9, 364 degrees of freedom. This gives a conservative 5% level of significance for the overall test because there are 105 comparisons among the samples. As in the previous analysis, all the samples differed with the exception of *L. saxatilis* at Golden Cap and St Margaret's at Cliffe (F=2·451, P=0·0102) and at Portland Bill and St Catherine's Point (F=2·271, P=0·0174). Table 2 shows the coefficients making up the first three standardized canonical variates. Whorl width one is evidently very important, being opposed to operculum area and aperture length on canonical one and to columella length on canonical two. On canonical three, aperture width is opposed to operculum area.

Table 2. *Coefficients forming standardized canonical vectors for the first three canonical variates,* L. saxatilis *and* L. arcana

AA, apical angle; AL, aperture length; AW, aperture width; CL, columella length; LL, lip length; OA, operculum area; SW, shell width; WW1, whorl width one; WW2, whorl width two.

Canonical 1 46%		Canonical 2 22%		Canonical 3 13%	
OA	-1.47	CL	-10.16	AW	-4.87
AL	-1.41	AA	-1.16	WW2	-0.72
AW	-1.27	AW	-0.65	AA	-0.61
SW	-0.77	LL	-0.19	SW	-0.08
AA	0.55	WW2	0.59	CL	-0.04
CL	0.66	OA	0.61	LL	0.05
WW2	0.70	AL	1.29	WW1	0.86
LL	1.46	SW	2.45	AL	1.51
WW1	2.65	WW1	5.50	OA	3.62

Inspection of Tables 1 and 2 suggests similarity between some vectors in terms of the rank order of the coefficients, with vectors one and, to some extent, two (Table 1) being displaced to two and three respectively in Table 2. This was investigated using the Spearman rank correlation coefficient, r_s. The values of the correlation coefficients are as follows:

vector 1, Table 1 : vector 2, Table 2, r_s=0·95, P<0·01
vector 2, Table 1 : vector 3, Table 2, r_s=-0·75, P<0·05.

Strong negative correlations, indicating the same ordering of the variables in the vectors but with opposite sign, are taken here to indicate vectors which are products of
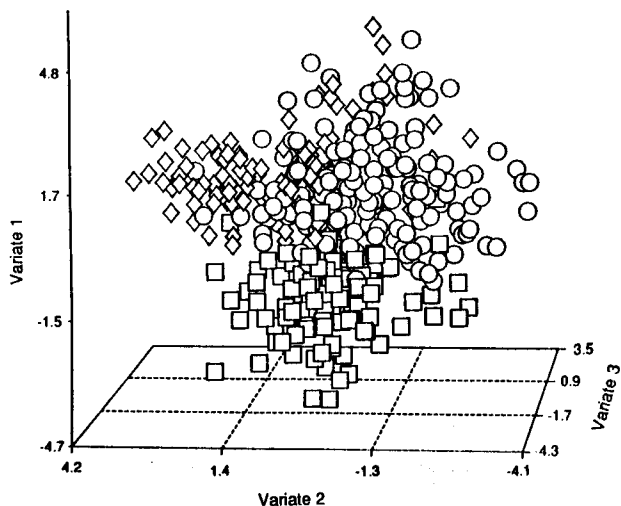
Figure 6. Canonical variate scores of the individual shells of *L. saxatilis* (○ , allopatric from *L. arcana*; ◇, sympatric with *L. arcana*) and *L. arcana* (□) plotted on the first three canonical variates.

the same combinations of variables. In other words it is the order of the variables which is important in comparing the vectors.

Figure 6 shows the canonical variate scores for the individual shells plotted on the first three variates in the analysis for both species. The two groups of *L. saxatilis* now separate on canonical variate two (remembering that this was identified with variate one in the analysis of *L. saxatilis* alone - see above), while the two species separate on canonical variate one. Variate three (weakly identified with two in the first analysis) does not produce a meaningful separation of groups of samples. The analysis was repeated keeping the sexes separate so as to determine whether *L. arcana* at Great Castle Head showed evidence of sexual dimorphism. The F statistic for difference between the sexes was only significant at $P=0.0707$, thus sustaining the null hypothesis of no significant difference.

We may conclude that the analyses have identified features of the shells which tend to separate two groups of *L. saxatilis* (sympatric and allopatric with respect to *L. arcana*) primarily on the basis of the relationship between columella length and whorl width one (*i.e.* the relative globosity of the shell), and further to separate the two species mainly on the basis of the relationship between operculum area and whorl width one.

These three groups were further examined using discriminant analysis, in which the discriminant functions calculated from the data were used to classify each individual with respect to the defined groups of individuals.

The results of this are shown in Table 3. The classification of *L. arcana* is very reliable, with approximately equal proportions being mis-classified to either allopatric or sympatric *L. saxatilis*. Sympatric *L. saxatilis* are also quite reliably classified, with most of the mis-classifications going to allopatric *L. saxatilis*. However, allopatric *L. saxatilis* are much less reliably classified, with about 60% of the mis-classifications being assigned to sympatric *L. saxatilis*.

Table 3. *Discriminant classification of shells*

| | Percentages classifying into the three categories: | | | |
	L. arcana	*L. saxatilis* (allopatric)	*L. saxatilis* (sympatric)	N
Observed category:				
L. arcana	91.2	3.9	4.9	102
L. saxatilis (allopatric)	12.4	69.2	18.3	169
L. saxatilis (sympatric)	2.6	11.2	86.2	116

Analysis of covariance

The relationships between the variables shown to be important in separating groups of rough periwinkles were further investigated using analysis of covariance (AN-COVA). Considering all 12 samples of *L. saxatilis* first, an ANCOVA was carried out using whorl width one as the dependent variable and columella length as the independent variable. The null hypothesis (H_0) of homogeneous slopes ($b_1=b_2 ... =b_{12}$) was rejected (P=0·0225), which violates a principal assumption of ANCOVA, so that while there was evidence of differences among the intercepts (H_0 $a_1=a_2 ... =a_{12}$ rejected, P=0·0001), this was difficult to interpret. The individual regressions were inspected, showing that the most divergent values among the slopes of the regression lines were those for Trevaunance (cliff sample), b=1·1280, and Golden Cap, b=1·0593; pooled regression value of b=0.9523). The data for these samples were removed sequentially and the analysis repeated each time. Removing just the Trevaunance sample still left heterogeneity among the slopes, removing the Golden Cap sample as well had the result that the null hypothesis of homogeneous slopes was not rejected (P=0·8430) and again the intercepts showed differences (P=0·0001). Table 4 shows the least squares means for whorl width one together with the probability levels associated with the null hypothesis that mean$_i$=mean$_j$. While the values for the sympatric samples are generally higher than those for the allopatric ones, Trevaunance (boulders) has a relatively low value, while that from Cae-du has a relatively high value. Since there are 45 tests in the Table, the critical probability level corresponding to P=0·05 is (0·05/45)=0·0011. On this criterion, the least squares means show many cases of significant differences. The least squares mean for Duckpool is significantly different from all of the other samples, both sympatric and allopatric. The values for Great Castle Head and Prawle Point animals are the same as for Trevaunance (boulders) and differ significantly from all of the allopatric samples except that from Cae-du. Furthermore, the value for Trevaunance (boulders) is not significantly different from any of the allopatric samples, while that for Cae-du is significantly different from all other allopatric samples except that from St Catherine's Point. Nevertheless, it is generally true that among the sympatric samples the least squares means are higher than among the allopatric ones (Mann-Whitney U=1, P=0·010), and it thus seems likely that there are two different groups, based on the relative width of whorl one: this is larger in *L. saxatilis* when sympatric with *L. arcana*. Apart from the exceptionally high value for Duckpool, all of the anomalies in Table 4 relate to the samples from Trevaunance (boulders) and Cae-du. It should be noted that the sample size for the former was extremely small (n=7).

Table 4. *Least squares means for whorl width one in the ANCOVA for this variable on columella length in* L. saxatilis
Also shown are the probabilities of obtaining a larger t, by chance, when $mean_i = mean_j$. The 5% level of significance corresponds to $P = 0.0011$ (see text for details).

	LS mean	1	2	3	4	5	6	7	8	9	10
Duckpool (sympatric) (1)	0.755	-	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Great Castle Head (sympatric) (2)	0.733		-	0.1802	0.0727	0.0043	0.0001	0.0001	0.0001	0.0001	0.0001
Prawle Point (sympatric) (3)	0.727			-	0.6277	0.0347	0.0003	0.0001	0.0001	0.0001	0.0001
Cae-du (allopatric) (4)	0.725				-	0.0717	0.0019	0.0001	0.0003	0.0001	0.0001
Trevaunance, boulders (sympatric) (5)	0.713					-	0.9007	0.3925	0.3314	0.0621	0.0278
St Catherine's Point (allopatric) (6)	0.712						-	0.2555	0.2149	0.0037	0.0012
Portland Bill (allopatric) (7)	0.707							-	0.8270	0.1376	0.0615
Alum Bay (allopatric) (8)	0.706								-	0.2739	0.2149
St Margaret's at Cliffe (allopatric) (9)	0.700									-	0.6299
St Alban's Head (allopatric) (10)	0.698										-

Table 5. *Least squares means for whorl width one in the ANCOVA for this variable on operculum area in* L. saxatilis *and* L. arcana. Also shown are the probabilities of obtaining a larger t, by chance, when mean$_i$=mean$_j$. The 5% level of significance corresponds to P=0.00075 (see text for details).

	LS mean	1	2	3	4	5	6	7	8	9	10	11	12
L. saxatilis (sympatric) Trevaunance boulders (1)	0.806	-	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
L. saxatilis (sympatric) Great Castle Head (2)	0.754		-	0.5288	0.0324	0.0154	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
L. saxatilis (allopatric) Prawle Point (3)	0.749			-	0.1265	0.0783	0.0020	0.0003	0.0001	0.0001	0.0001	0.0001	0.0001
L. saxatilis (allopatric) Cae-du (4)	0.739				-	0.7217	0.0898	0.0166	0.0001	0.0001	0.0001	0.0001	0.0001
L. saxatilis (sympatric) Duckpool (5)	0.736					-	0.2069	0.0475	0.0005	0.0005	0.0001	0.0001	0.0001
L. saxatilis (allopatric) Portland Bill (6)	0.725						-	0.3996	0.0372	0.0317	0.0001	0.0001	0.0001
L. saxatilis (allopatric) Alum Bay (7)	0.718							-	0.3542	0.3054	0.0001	0.0001	0.0001
L. saxatilis (allopatric) St Catherine's Point (8)	0.710								-	0.8753	0.0001	0.0001	0.0001
L. saxatilis (sympatric) Trevaunance cliff (9)	0.709									-	0.0001	0.0001	0.0001
L. arcana Prawle Point (10)	0.680										-	0.0877	0.0360
L. arcana Great Castle Head (11)	0.669											-	0.3249
L. arcana Trevaunance cliff (12)	0.662												-

Considering next all 15 samples and the relationship between whorl width one and operculum area, and treating the latter as the independent variable, again H_0, $b_1=b_2$ ... $=b_{15}$, was rejected (P=0·0003) while there was evidence of differences between intercepts. It was now necessary to remove three data sets, namely *L. saxatilis* at Golden Cap, St Alban's Head and St Margaret's at Cliffe, in order to obtain homogeneity among the slopes (H_0, P=0·1792). Table 5 shows the least squares means and probability levels for equality. In this case the critical probability level corresponding to P=0·05 is 0·0008 (*i.e.* 0·05/66). This criterion separates *L. arcana* from all the *L. saxatilis* samples, indicating a relatively much smaller whorl width one in relation to operculum area in *L. arcana*. This distinction remains true if the least squares means for the samples from Golden Cap, St Alban's Head and St Margaret's at Cliffe, which were excluded on the grounds of heterogeneous regression slopes, are included in the ranking. The values for the least squares means of all of the *L. saxatilis* samples differ significantly from those of all of the *L. arcana* samples. The values of the *L. arcana* samples do not differ from each other, although there are some significantly different values within the *L. saxatilis* samples. The relatively poor distinction between sympatric and allopatric *L. saxatilis* (Table 5) would be expected since the whorl width one : operculum area relationship differentiates primarily between the two species (Figure 6).

Table 6. *Least squares means for foot area in the ANCOVA for this variable on shell width in* L. saxatilis *and* L. arcana

Also shown are the probabilities of obtaining a larger t, by chance, when mean$_i$=mean$_j$. The 5% level of significance corresponds to P=0.005 (see text for details).

	LS mean	1	2	3	4	5
L. saxatilis (allopatric) Golden Cap (1)	1.219	-	0.6666	0.0113	0.0015	0.0001
St Margaret's at Cliffe (2)	1.211		-	0.0176	0.0021	0.0001
L. arcana Great Castle Head (3)	1.169			-	0.3297	0.0005
L. saxatilis (allopatric) Portland Bill (4)	1.150				-	0.0305
L. saxatilis (sympatric) Great Castle Head (5)	1.110					-

Finally we have investigated the relationship of foot area to the shell variables. Because measurements of foot area are comparatively time consuming our information is restricted to Great Castle Head (for both species), Golden Cap, Portland Bill and St Margaret's at Cliffe. All the variables measured in this study show a high correlation with one another. We used the STEPWISE procedure in SAS (SAS Institute Inc., 1985) on data from these shores to find the single shell variable which was the best predictor of foot area, which was shell width. The largest coefficient for shell width in Tables 1 and 2 is on canonical 3 (Table 1). However, it is also important on canonical 1 (Table 1)

and canonical 2 (Table 2), which are regarded as being the same variate in the two analyses and the one principally involved in the separation of the two forms of *L. saxatilis*.

ANCOVA, using foot area as the dependent and shell width as the independent variable, retained H_0 for equal slopes (P=0·2167) and rejected H_0 for equal intercepts (P=0·0001). Table 6 shows the least squares means and probability levels for $mean_i = mean_j$. The critical probability level is 0·005 (*i.e.* 0·05/10). On this criterion, the allopatric *L. saxatilis* populations at Golden Cap and St Margaret's at Cliffe together with *L. arcana* at Great Castle Head have a significantly greater foot area for a given shell width than the sympatric Great Castle Head *L. saxatilis* population. Also the allopatric *L. saxatilis* at Golden Cap and St Margaret's at Cliffe have a relatively greater foot area than the population at Portland Bill, although the last is not significantly different from *L. arcana* at Great Castle Head. Furthermore, although the least squares mean is lowest for sympatric *L. saxatilis* at Great Castle Head, the difference between this population and the allopatric *L. saxatilis* at Portland Bill is not significant. These relationships are expressed in Figure 7.
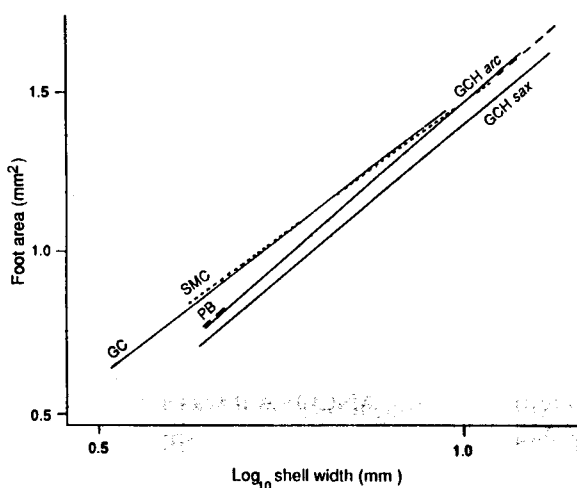

Figure 7. Lines for the regression of foot area on shell width in four populations of *L. saxatilis* (GCHsax, Great Castle Head; GC, Golden Cap; PB, Portland Bill; SMC, St Margaret's at Cliffe) and one of *L. arcana* (GCHarc, Great Castle Head).

DISCUSSION

When *L. saxatilis* and *L. arcana* occur on a shore together we have referred to them as 'sympatric'; when *L. saxatilis* occurs by itself we have referred to it as 'allopatric from *L. arcana*'. We have not yet observed *L. arcana* on a shore without *L. saxatilis* also being present. Obviously the periwinkles are accompanied on shores by a variety of other gastropods. However it is the two species *L. saxatilis* and *L. arcana* which are striking in their degree of overlap on shores where they both occur (Grahame & Mill, 1986).

Canonical variate analysis has identified the variables of columella length and whorl width one (see Figure 2) as the most important of those measured in separating samples

of *L. saxatilis* (Figures 3 & 5; Table 1), accounting for 33% of the total variation in the samples. These characters together indicate the relative globosity of the shell and the vector concerned tends to separate the samples depending upon whether or not they are sympatric with *L. arcana*. Indeed an analysis of covariance (ANCOVA) demonstrated that, in sympatry, whorl width one becomes relatively larger - *i.e.* the shell becomes more globose. This analysis showed the differences to be usually highly statistically significant. The remaining canonical vectors in the *L. saxatilis* analysis (Table 1) identify columella length and operculum area/aperture length (canonical two) or shell width (canonical three) as variables contributing to separation of the samples, but there is as yet no explanation of the possible significance of these separations.

We have considered the possible effects of any sexual dimorphism for the samples in which males were included (Figure 5); it has no effect on the above conclusions.

As was noted above, the samples from Trevaunance (boulders) and Cae-du have, respectively, rather lower and higher least squares means for whorl width one on columella length. The value from the former may be unreliable as it was based on an extremely small sample size (n=7). However, the value for Cae-du is interesting since this shore is in Cardigan Bay, to the north of the zone where *L. saxatilis* and *L. arcana* are sympatric, whereas the other allopatric sites are all to the east of this zone. It may be that allopatric *L. saxatilis* in Cardigan Bay have different characteristics from those along the south coast of England, introducing the possibility of a larger scale geographical cline of variation. There is no indication that such a cline (if it exists) is contributing to the observed changes in *L. saxatilis* when sympatric with *L. arcana*. The possibility of a cline will be investigated further in future work.

When *L. arcana* was included in the analysis the same separation of *L. saxatilis* samples along a 'globosity' vector was observed, but this was now the second most important canonical variate. The first was identified as a whorl width one: operculum area variate, accounting for 46% of the total explained variation and producing a species separation (Figure 6). Using ANCOVA it was shown that the whorl width one of *L. arcana* from the shores sampled is significantly smaller than that of *L. saxatilis*, but the *L. saxatilis* samples themselves did not fall into any obvious order.

Interspecific differences in shape have been noted in rough periwinkles before. For example, Hannaford Ellis (1979) found *L. arcana* to have a relatively larger aperture (more patulous) than *L. saxatilis* in north Wales, and Ward & Janson (1985) used canonical variates analysis to demonstrate interspecific differences on shores in Yorkshire. What is novel in the present work is that the relegation of canonical variate one to canonical variate two when *L. arcana* was included indicates that the interspecific differences are greater than the intraspecific differences between allopatric and sympatric samples of *L. saxatilis*. Furthermore, discriminant analysis demonstrated that *L. saxatilis* in allopatry is more likely to be classified as '*L. arcana*' than is *L. saxatilis* in sympatry. That is, where the two species occur together, they are more distinct.

We shall now consider the biological significance of these features. In the case of different *L. saxatilis* populations, the shift in size of whorl width one towards increased shell globosity in populations sympatric with *L. arcana* is not of obvious biological

significance. We suggest that perhaps it may indicate a change in brood size, which is reflected in shell shape since the brood must be accommodated in the lower part of the shell when the animal is withdrawn. In making this suggestion we note that Hart & Begon (1982) found the relative weights of broods to differ in *L. saxatilis* populations from two different habitats on the same shore, attributing this to a change in selective regime between populations in boulders (smaller clutch weights) and in crevices (larger clutch weights). For the only shore from which we have analysed separate cliff and boulder shell samples (Trevaunance), inequality of the slopes of the regression lines of whorl width one on columella length makes comparison of the relative size of the whorl width impossible. As we have no data on soft parts with which to test this hypothesis, it remains to be examined in future work.

In the case of differences between the species, the relative sizes of operculum area, aperture length and, to some extent, aperture width have been identified as discriminant variables of roughly equal weight and negative sign, opposed to whorl width one. Grahame & Mill (1986) showed that on one shore (on which Great Castle Head was the 'exposed' site) foot area might be ecologically important to *L. saxatilis* and *L. arcana*, the latter having a greater area. Here we have shown that foot area and shell width are closely related, and that *L. saxatilis* from Great Castle Head had the smallest relative foot areas in the samples studied. Furthermore, the relative foot areas in *L. saxatilis* from Great Castle Head are also smallest when related to operculum area, aperture length, aperture width and whorl width one. Although there is substantial variation among the *L. saxatilis* populations in this study, it is striking that at Great Castle Head in sympatry with *L. arcana* the foot area of *L. saxatilis* should be so small. This seems unlikely to be merely coincidental, since the animals from the other sympatric shores are also likely to have a very small foot area. We suggest this because the shells from these shores most closely resemble those from Great Castle Head (Figures 3 & 4; Table 3), and foot area is closely related to shell shape.

However, the likely adaptive significance of shell shape differences between the species remains to be examined in detail. The high intercorrelation of the variables (multicolinearity) has presented difficulties in interpreting the data; future work will be directed at solving the above problems.

We draw three principal conclusions. The first is that nearly all of the populations differ significantly between shores. The second is that, despite substantial intraspecific variation and some interspecific overlap, the species *L. saxatilis* and *L. arcana* in populations on exposed shores may be distinguished by shell shape. The third is that among *L. saxatilis* populations on such shores there is evidence of change in shape depending on whether or not the animals are sympatric with *L. arcana*.

Although local variation on a single shore has been demonstrated (Hart & Begon, 1982; Janson & Sundberg, 1983; Grahame & Mill, 1986), nevertheless the between-shore variations described in this paper are such as to indicate more widespread phenomena, particularly with regard to exposed shore *L. saxatilis*. For instance it appears that either the presence of *L. arcana* in some way affects the shape of *L. saxatilis*, or there is some other factor which (i) induces a shape change in *L. saxatilis* and (ii) independently allows the presence of *L. arcana*. The former seems to be the more parsimonious

hypothesis, and therefore we suggest that there is evidence of character displacement in exposed shore *L. saxatilis* when it is sympatric with *L. arcana*. This in turn implies that there must be a degree of interspecific competition between these two prosobranchs. Further work will address the question of what environmental factors underlie the features of shape discussed in this paper.

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