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Journal of Shellfish Research, Vol. 20, No. 1, 501–508, 2001.

CORRELATION OF MORPHOLOGICAL DIVERSITY WITH MOLECULAR MARKER DIVERSITY IN THE ROUGH PERIWINKLE *LITTORINA SAXATILIS* (OLIVI)

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ABSTRACT Two morphological varieties of *Littorina saxatilis*, widespread around the United Kingdom, are a thin-shelled, high-shore morph (*L. saxatilis* H) and a thick-shelled, mid-shore animal (*L. saxatilis* M). Mitochondrial DNA (mtDNA) analysis by PCR-RFLP was used to test whether gene flow between these morphs is restricted. At Galloway, Scotland, replicated sampling (different years and different transects over a distance of 800 m) has been undertaken. One mtDNA haplotype is predominant in H and a different haplotype common in the M animals. Repeatability in space and time suggests a real H/M differentiation. A similar pattern of mtDNA haplotype variation is seen in a single sample of the Swedish E and S morphs of *Littorina saxatilis*. However, this pattern is not evident everywhere. Variation in the mtDNA and four nuclear DNA loci was examined within and between *L. saxatilis* H and M morphs from the south coast of England. Because shape variation in this region additionally separates into three shape groupings (regions identified from multivariate morphometric analysis where shape is more homogeneous within, than between groups), genetic variability was examined within and between these groupings as well as between H and M. On the south coast, an apparent association of shape and mtDNA haplotype is identified, but AMOVA analysis shows no support for the association being with shape grouping or H and M morphs. Although the nature of this shape-genotype association is unknown, a mtDNA haplotype and an allele at the nuclear CAL-2 locus are confined mainly to one shape group. Analysis of association of mtDNA haplotype with H and M morphology suggests a strong correlation can be found in some areas (Galloway, Mumbles (south Wales), and between similar morphs in Sweden) yet no association is seen at others (Ravenscar, UK, Ballynahown, Ireland, and the south coast of England). Thus, unravelling the basis of the H and M forms will require more detailed studies, with replication, as at Galloway, and also with additional molecular markers.

KEY WORDS: AMOVA, *Littorina saxatilis*, mitochondrial DNA, morphometrics, PCR, RFLP, shape

INTRODUCTION

Shell shape in gastropod mollusks is known to be affected by environmental factors. Selection as a result of crab predation or stone damage can produce heavy shells with small apertures, while animals in exposed conditions where crab damage is minimal, but risk of dislodgement by waves is high, tend to have thin shells with wide apertures (Boulding 1990). In *Littorina saxatilis* (Olivi) two forms typical of these morphological extremes are widespread. Hull et al. (1996) referred to the thin-shelled, patulous form, found in high-shore areas as *L. saxatilis* H, and the thick-shelled variant that occurs in the mid-shore as *L. saxatilis* M. Although the basis of this shell polymorphism undoubtedly has a strong environmental component, evidence from embryological characters (Hull et al. 1996) and detection of assortative mating for these morphotypes (Hull 1998, Pickles & Grahame 1999) suggests that there may be a genetic component to the differentiation of *L. saxatilis* H and M. However, the only molecular level study of genetic differentiation between these forms was limited to two sites on the east coast of Yorkshire, England. Random amplified polymorphic DNA (RAPD) analysis showed differing degrees of differentiation at these two sites (Wilding et al. 1998). No wide scale geographic studies have yet been undertaken. This is important since such evidence is necessary to determine the extent of gene flow between these morphs, which is critical for the understanding of their relationships.

Mitochondrial DNA (mtDNA) is a useful molecular marker for such a study because of its high rate of evolution (Avise 1994), and a substantial portion of the *L. saxatilis* mitochondrial genome has been sequenced (Wilding et al. 1999), thus permitting the targeting of primers. Population genetic studies of gastropods have often relied on mitochondrial markers (Kirby et al. 1997, Hellberg 1998, Kyle & Boulding 1998) and Wilding et al. (2000a) have recently examined mtDNA cytochrome oxidase I (CoI) variation in UK and Irish *Littorina*. We also have developed primers for the amplification of four variable nuclear DNA (nDNA) loci in *Littorina saxatilis* (Wilding et al. 2000b).

Here shape variation and genetic variation are examined at four levels. (1) Between *L. saxatilis* H and M in replicated samples from southwest Scotland. (2) Between *L. saxatilis* H and M on the south coast (where nDNA variation is also examined). (3) Between *L. saxatilis* H and M at other locations. (4) Along the south coast of the United Kingdom where three 'shape groupings' are recognized. It is shown that both shape and mitochondrial DNA do vary geographically, but that there is only a limited correlation of the two. There is however evidence for differentiation at the mtDNA level between *L. saxatilis* H and M in some areas of the UK.

MATERIALS AND METHODS

Animals were collected from 17 sites around the UK (Table 1). Images of the shells were captured with a digital camera and used for morphometric measurements. Measuring of shell images was performed using Sigma Scan to measure eight shell variables (Fig. 1). These shell variables were standardized for size using the geometric mean transform (Reist 1985) and then submitted to a canonical variate analysis in SAS v 6.0 (SAS Inc. 1990). The shell was subsequently crushed and a portion of the head-foot removed for DNA extraction using the single-fly DNA extraction protocol of Ashburner (1989). DNA concentration was measured by fluorometry and adjusted to 10 ng.μl^{-1}. A 967 bp segment of mitochondrial DNA spanning the cytochrome oxidase I–cytochrome

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TABLE 1.

Collection sites for samples of *L. saxatilis* H and M. South Coast samples are grouped in the three shape zones used in some analyses.

Collection site	British National Grid Reference	*N* (H)	*N* (M)
South coast			
Kent			
St Margaret's at Cliffe	TR368444	10	10
Folkestone	TR245369(H) & TR244373(M)	10	9
South-central			
St Alban's Head	SY959754	10	
Portland Bill	SY675683	9	
East Fleet	SY799635	10	
The Fleet (gravel)	SY758665		10
Golden Cap	SY407918		20
Pinhay Bay	SY318907		5
South-west			
Cargreen	SX436627		10
The Lizard	SW699114	10	
Trevaunance	SW725519	10	3
Other locations			
Galloway			
St. Ninian's Cave	NW417364	48†	49†
Back Bay	NW368394	7	10
South Wales			
Mumbles	SS632873	10	10
North East England			
Ravenscar	NZ984021	10	10
Ireland			
Ballynahown	IGR: L 992202	10	10
Sweden			
Ursholmen	58'50"10°N 0'59"4°E	9*	8*

N = sample size.
† replicated samples taken at Galloway, see Fig. 4.
* morphs in Sweden are E and S, not H and M—see text.
IGR = Irish Grid Reference.

oxidase II (CoI-CoII) gene boundary (Wilding et al. 1999) was then amplified using the primers saxCoI and saxCoII (Wilding et al. 2000a). PCR was performed in 50μl volumes containing 50 mM KCl, 10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 200 μM each dNTP, 25 pmol each primer, 25 ng DNA and 1U *Taq* (Super*taq*, HT Biotechnologies). PCR

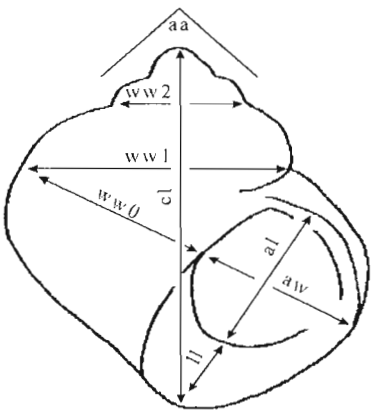

Figure 1. The eight shell variables measured in Sigma Scan. aa = apical angle. al = aperture length. aw = aperture width. cl = columellar length. ll = lip length. ww 0–2 = whorl width 0–2.

conditions of 1 × 94°C, 5 min; 35 × (94°C, 1 min, 55°C, 30 secs, 72°C, 1 min); 1 × 72°C, 5 min were employed. In an initial study, 400 bp of mtDNA were sequenced in 50 individuals from 18 populations (Wilding et al. 2000a). From the resultant sequences variable restriction sites were deduced. Variation was then assessed by RFLP using three restriction enzymes — *Dde*I, *Dra*I and *Hin*dIII (*Dra*I is not variable in *L. saxatilis* but is in other rough periwinkles). Restriction enzyme digestion was undertaken in 15 μl volumes containing 3-5U enzyme in 1X buffer (supplied with enzyme) and 5 μl PCR product. Following digestion, restriction fragments were separated on 2% agarose gels. Variant patterns generated by each enzyme were labeled A, B, C *etc.* and a three letter composite haplotype describes the variation in each animal. An analysis of molecular variance (AMOVA: Excoffier et al. 1992) was used to examine genetic variation from animals along the south coast of England. *Littorina saxatilis* in this region is known to exhibit substantial shape variation which has been shown to partition into three groups (Grahame and Mill 1992, Mill and Grahame 1995), a south-west, a south-central, and a Kent grouping (Fig. 2). Upon this broad-scale variation there is superimposed the H and M differentiation. AMOVA was used to quantify the partitioning of mtDNA variation into a 'shape grouping' component and a 'H and M' component. If substantial variability is accounted for by either of these then the expectation is that these shape differences are indicative of underlying genetic differentiation. If little genetic variability is partitioned into either of these compo-

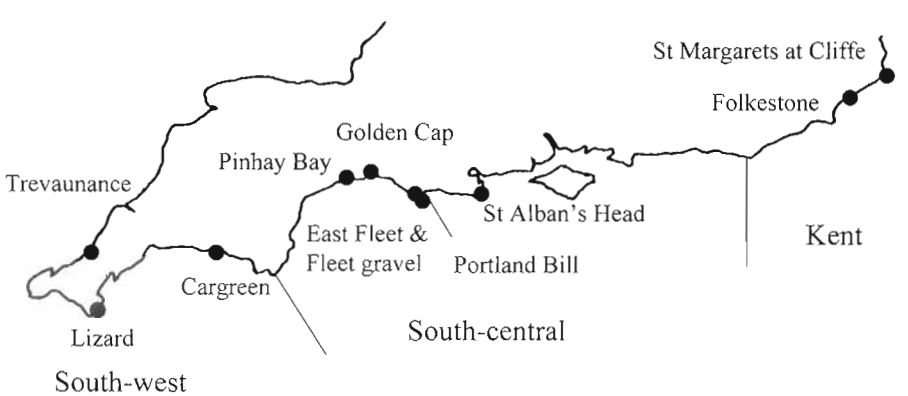

Figure 2. Delineation of shape groupings as described by Grahame and Mill (1992) and Mill and Grahame (1995).

nents then there is no detectable genetic differentiation at the mitochondrial DNA level between these groups. AMOVA was performed in ARLEQUIN v1.1 (Schneider et al. 1997).

In addition to the mtDNA analysis, patterns of nuclear DNA (nDNA) polymorphism have been examined in these animals (Wilding et al. 2000b). Four nuclear loci comprised of two calmodulin introns (CAL-1 and CAL-2), and two anonymous loci (X80 and DELETION) were examined for variation. All nDNA PCR reactions were undertaken in 25 μl of 50 mM KCl, 10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 200 μM each dNTP, 12.5 pmol each primer, 12.5 ng DNA and 0.5 U *Taq* (Super*taq*, HT Biotechnologies). PCR cycling conditions were 1 × 94°C, 5 min; 35 × (94°C, 1 min, 55°C, 30 secs, 72°C, 1 min); 1 × 72°C, 5 min, although for CAL-1 the annealing temperature was increased to 57°C, and DELETION in which annealing was at 57°C and the extension time was reduced to 30 seconds. CAL-1 and CAL-2 polymorphism was examined by *Taq*I and *Dde*I digestion respectively and in X-80 with *Msp*I. Restriction digestion was performed at the appropriate temperature for 2 hours and products were separated on 2% agarose gels. For the DELETION locus, alleles differed due to length variation and this was recognized after PCR products were run on 3% agarose gels. These nDNA data were not suitable for AMOVA analysis (because although the genetic distance between genotypes was calculable for those loci examined by RFLP, the mutational distance between alleles at the length variable DELETION locus was not. Thus the relationships among multi-locus genotypes, necessary for AMOVA analysis, were unclear). Correlation of shape variation with molecular variation was therefore examined using a Mantel test employed in NTSYS through comparison of the population matrix of genetic distance (different genetic distance measures were tested in case of subtle differences due to method employed) with the Mahalanobis distance matrix.

RESULTS

Mitochondrial DNA Variation

A neighbor-joining tree based on all sequences of CoI-CoII from *Littorina* shows two groupings of *L. saxatilis* (Fig. 3). Although there are 13 separate *L. saxatilis* sequences, these cannot all be distinguished using RFLP since there are many variable positions at this locus where there is no restriction enzyme with a recognition sequence spanning the site. However, three enzymes (*Hin*dIII, *Dra*I and *Dde*I) do recognize variable positions in this sequence within the rough periwinkles (Wilding et al. 2000a), although *Dra*I is not variable in *L. saxatilis*. In *L. saxatilis*, *Hin*dIII produced two patterns and *Dde*I four patterns. The four composite haplotypes encountered in *L. saxatilis* were designated AAA, ADA, BBA and BCA.

The most pronounced evidence for association of mitochon-

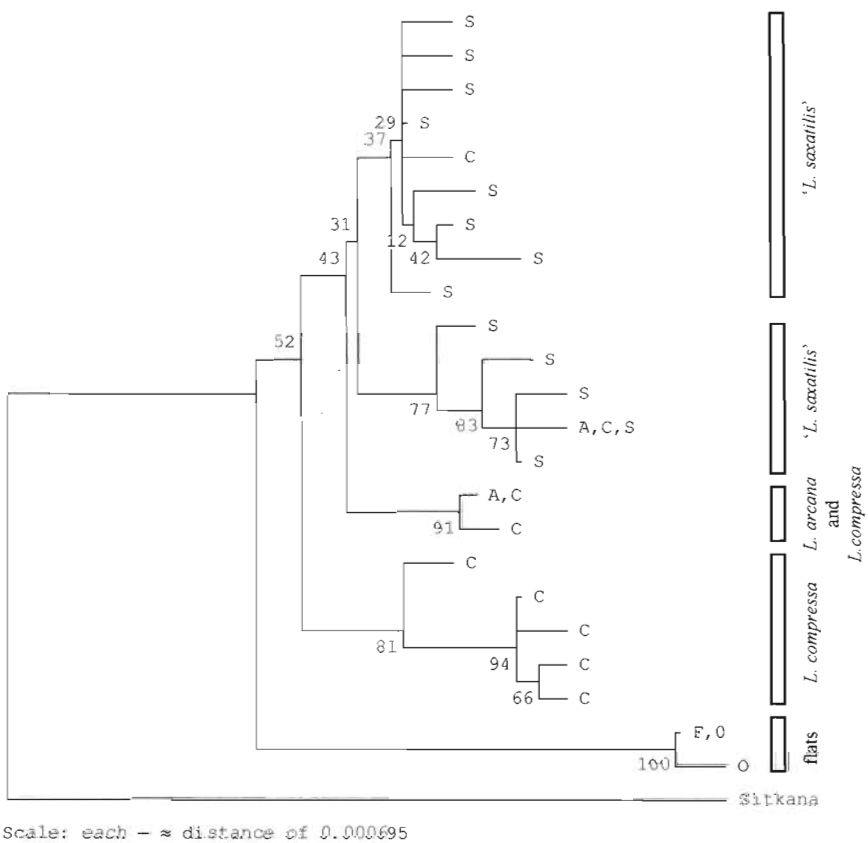

Figure 3. Phylogeny of rough periwinkle CoI sequences (adapted from Wilding et al. 2000a) using neighbor-joining of Jukes-Cantor distances. S = *L. saxatilis*, A = *L. arcana*, C = *L. compressa*, F = *L. fabalis* and O = *L. obtusata*. The two divisions of *L. saxatilis* sequences are denoted, note that some *L. arcana* and *L. compressa* sequences are shared with *L. saxatilis* or cluster in these "*L. saxatilis*" groups. Flats = sequences from the flat periwinkles *L. fabalis* and *L. obtusata*. The outgroup is *L. sitkana*. Numbers on the nodes are bootstrap values from 100 pseodoreplicates.

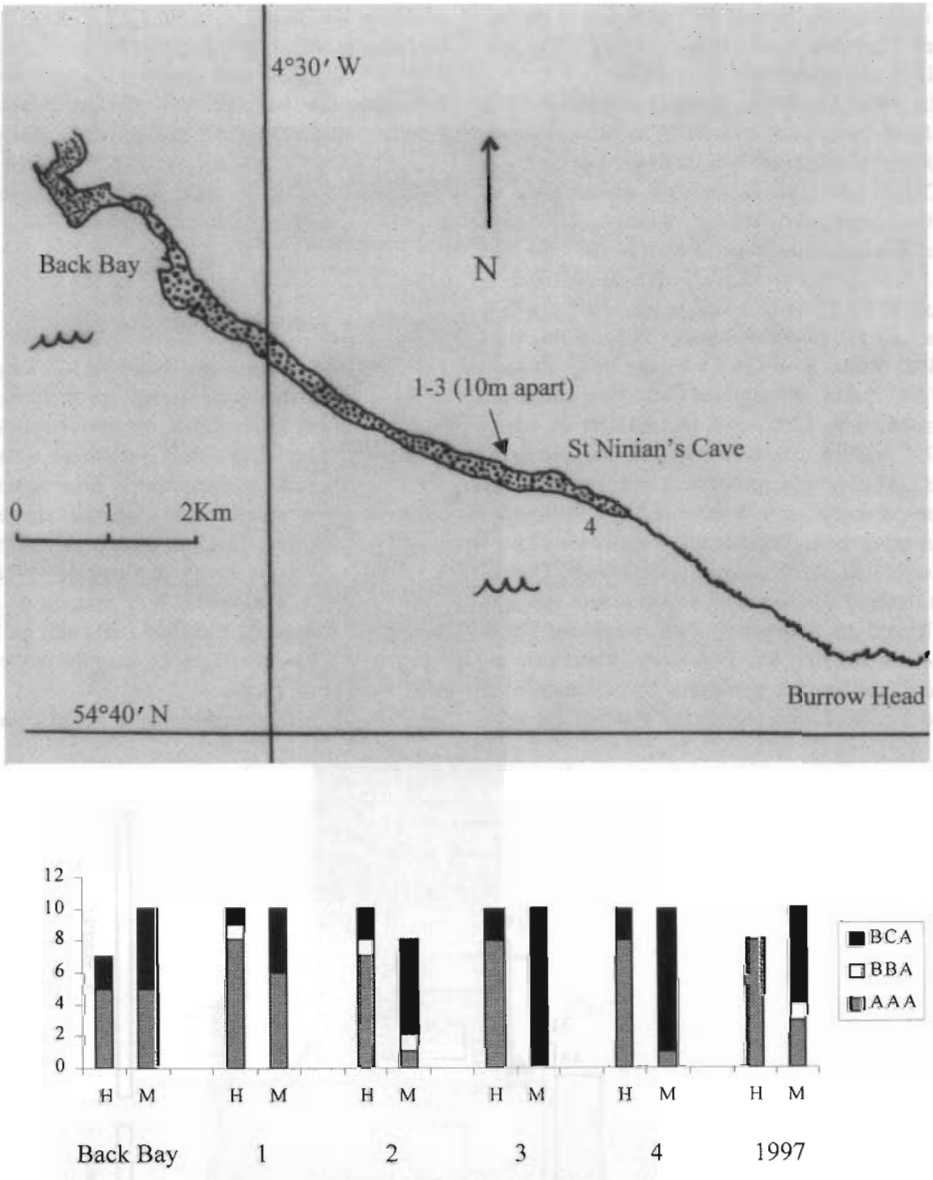

Figure 4. Distribution of mtDNA haplotypes in separate transects, undertaken in 1997 and 1998, at Galloway, southwest Scotland. Sites 1–3 were spaced 10 m apart and are separated from site 4 by approximately 800 m of shingle beach. The 1997 sample was taken from the same site as site 3 of 1998.

drial haplotype with morphology is found at Galloway in southwestern Scotland. Here one collection in 1997 and five collections in 1998 (from different transects) were typed. The AAA haplotype was predominant in *L. saxatilis* H and the BCA haplotype in *L. saxatilis* M (Fig. 4). Additional data from Mumbles (South Wales) also suggests differences between H and M, but at Ravenscar (east coast of England) and Ballynahown (western Ireland) no differences were detectable (Table 2). At Ursholmen, Sweden, differences were detected between the E and S morph of *L. saxatilis*. The E (exposed) morph, like *L. saxatilis* H, is wide apertured and thin-shelled and the S (sheltered) morph thick-shelled. However, unlike *L. saxatilis* H and M, where animals are separated vertically on the shore, E and S are separated locally, by habitat along the shore, being found in exposed and sheltered localities respectively. For the small sample examined, all nine E had the AAA haplotype, and 7 of 8 S had the BCA haplotype. These are the same haplotypes separating the H and M at Galloway.

South Coast of England — Morphological

Littorina saxatilis are shown to cluster into two groups on the basis of shell variation analyzed by canonical variate analysis (Fig. 5). When canonical variate means are plotted, the two groupings are associated with *L. saxatilis* H and M.

South Coast of England — Molecular

The distribution of haplotypes along the south coast within *L. saxatilis* H and M is shown in Table 2 and Fig. 6. There is sub-

TABLE 2.

Observed number of the 4 mtDNA haplotypes in the studied populations, and allele frequencies at the 4 nDNA loci for the south coast samples.

Collection site	MtDNA haplotype				CAL-1		CAL-2			X-80			Deletion		
	AAA	ADA	BBA	BCA	A	B	A	B	C	A	B	C	A	B	*N*
South coast															
South-east															
St Margaret's at Cliffe—H			10		0.45	0.55		1		0.2	0.8		0.3	0.7	10
St Margaret's at Cliffe—M	1		8	1	0.55	0.45		1		0.1	0.9		0.6	0.4	10
Folkestone—H			3	7	0.15	0.85		1		0.05	0.95		0.25	0.75	10
Folkestone—M	1		2	6	0.167	0.833		1			1		0.222	0.778	9
South-central															
St Alban's Head		1	9		0.6	0.4	0.4	0.6		0.15	0.85		0.35	0.65	10
Portland Bill		6	3		0.333	0.667	0.06	0.944			1		0.389	0.611	9
East Fleet		2	8		0.25	0.75		1		0.1	0.9		0.45	0.55	10
The Fleet (gravel)			10		0.4	0.6	0.1	0.9			1		0.3	0.7	10
Golden Cap		20			0.325	0.675	0.5	0.5		0.275	0.725		0.1	0.9	20
Pinhay Bay		5			0.8	0.2	0.8	0.2			1		0.4	0.6	5
South-west															
Cargreen	5			5	0.85	0.15		0.95	0.05	0.1	0.9		0.25	0.75	10
The Lizard		6		4	0.25	0.75	0.1	0.9		0.55	0.45			1	10
Trevaunace—H	1	1	6	2	0.4	0.6		1		0.2	0.75	0.05		1	10
Trevaunace—M			1	2	1			1		0.667	0.333		0.167	0.833	3
Other locations															
Galloway															
St. Ninian's Cave—H	39		2	7											48
St. Ninian's Cave—M	11		2	36											49
Back Bay—H	5			2											7
Back Bay—M	5			5											10
South Wales															
The Mumbles—H	3		5	2											10
The Mumbles—M			7	3											10
North East England															
Ravenscar—H	10														10
Ravenscar—M	10														10
Ireland															
Ballynahown—H	1		9												10
Ballynahown—M			10												10
Sweden															
Ursholmen—E	9														9
Ursholmen—S	1			7											8

N = sample size.

stantial variation within both H and M forms of *L. saxatilis* but no obvious difference in haplotypes between the two groups. However, there is some evidence for association of haplotype with shape grouping since the haplotype ADA is found mainly on the south coast in the south central group of shape variation. To investigate if haplotype partitioned with shape, haplotypes were plotted onto a canonical variate analysis of shape. The resultant, apparently random, distribution of haplotypes throughout the morphospace defined by the first three canonical variates shows little association (Fig. 7). Nevertheless, discriminant analysis with cross validation shows that the best classification on the basis of shape variables is back to the parent haplotype group (Table 3) indicating some underlying association of shape and haplotype. However, AMOVA analysis (Table 4) suggests that neither shape groupings nor H and M are the covarying factor.

Variation was also high in the nDNA dataset (Table 2: G_{ST} = 0.1601, H_T = 0.3447, H_S = 0.2895). Mantel comparisons of the morphological Mahalanobis distance (D^2) with Nei's (unbiased) distance and the Prevosti distance from the nuclear-DNA RFLP data showed that, irrespective of the genetic distance matrix employed there is no evidence of association of shape and genotype; p [random z < obs. z] = 0.337 and 0.345 for association with the Nei and Prevosti distance respectively. However, as was found for the mtDNA dataset there is an association between nDNA genotype and shape grouping in that the allele CAL-2^A is found at the highest frequency in the south-central region where the haplotype ADA is encountered.

DISCUSSION

It is apparent that around the coastline of the UK and Ireland there is a separation of *L. saxatilis* into a thin-shelled, patulous, high-shore form (*L. saxatilis* H), and a thick-shelled mid-shore

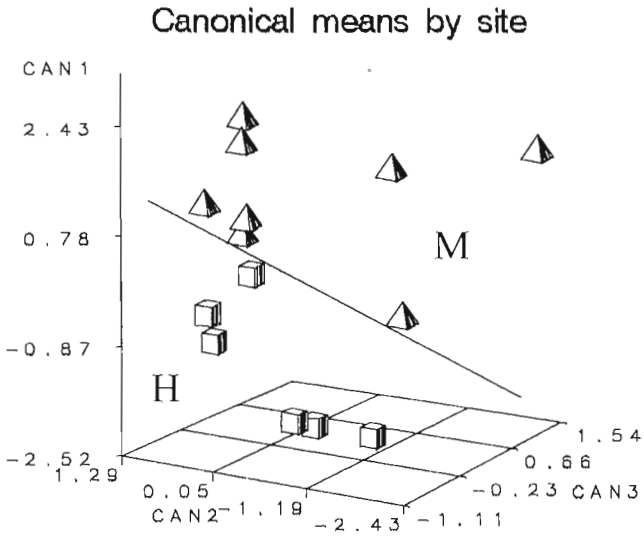

Figure 5. Population means from canonical variate analysis of south coast *L. saxatilis*.

form (*L. saxatilis* M). In this study we examined whether mitochondrial DNA variation provided evidence for genetic differentiation of these morphological varieties. At Galloway, Scotland, where repeated sampling on the shore has been undertaken, definite genetic differences are found between the morphs, with consistently different haplotype frequencies between H and M over both separate years and over multiple transects. Such repeatability shows the pattern is neither a temporal phenomenon, nor a simple distance effect. We found similar genetic differentiation between the Swedish E and S morphs of Janson (1982) which have similar morphologies to H and M. Interestingly, the differentiation was due to differing frequencies of the same haplotypes (high frequency of AAA in H/E and high frequency of BCA in M/S). Differentiation of mtDNA is also suggested at Mumbles (South Wales) where the frequency of AAA in *L. saxatilis* H is 0.3, compared to 0 in M — once again, it is the AAA haplotype which is involved. In contrast, no differentiation is indicated at Ravenscar or Ballynahown, but there is little mtDNA haplotype variation to partition at either of these sites.

On the south coast of England there are two obvious patterns to variation in *Littorina saxatilis* shell shape. On a broad scale, there are three "shape groupings" within which shape, analyzed by multivariate canonical variate analysis, is typically homogeneous but among which shape differs (Grahame & Mill 1992, Mill & Grahame 1995). Superimposed upon this, is the morphological distinction into H and M. Both mtDNA and nuclear DNA variation have been analyzed in samples from along the south coast to examine whether there is evidence that mtDNA differentiates H and M as at Galloway, and additionally if there is any evidence for restriction to gene flow between the shape groupings. Discriminant analysis of shape variation with mtDNA haplotypes as groups suggests a correlation between mtDNA haplotype and some aspect of shape on the south coast. However, there is no detectable association of mtDNA or nDNA with either shape groupings or H and M using AMOVA. Thus although a shape-mtDNA correlation has been detected it is not due to either of the *a priori* groupings considered here. In contrast to this, both an mtDNA haplotype and an nDNA allele at the CAL-2 locus are mainly limited to one of the shape groupings; the south central grouping of shape variation. It is likely that the AMOVA analysis of shape groupings does not detect this as a significant association due to the high variability of mtDNA within the shape groupings, effectively masking the 'between group' component. Nevertheless, the correlation of genetic differences at both the mitochondrial and nuclear DNA level with a known shape-group suggests that there is a real population difference.

Thus the pattern of genetic variation in *L. saxatilis* H and M is not simple. MtDNA evidence does not suggest that *L. saxatilis* H are consistently different from *L. saxatilis* M, although this can be the case on a particular shore. Given that these forms have different embryological characteristics (Hull et al. 1996) and display assortative mating (Hull 1998, Pickles and Grahame 1999) and that, in *Littorina*, there is a known substantial genetic component to shell shape (Grahame and Mill 1993), it is perhaps surprising

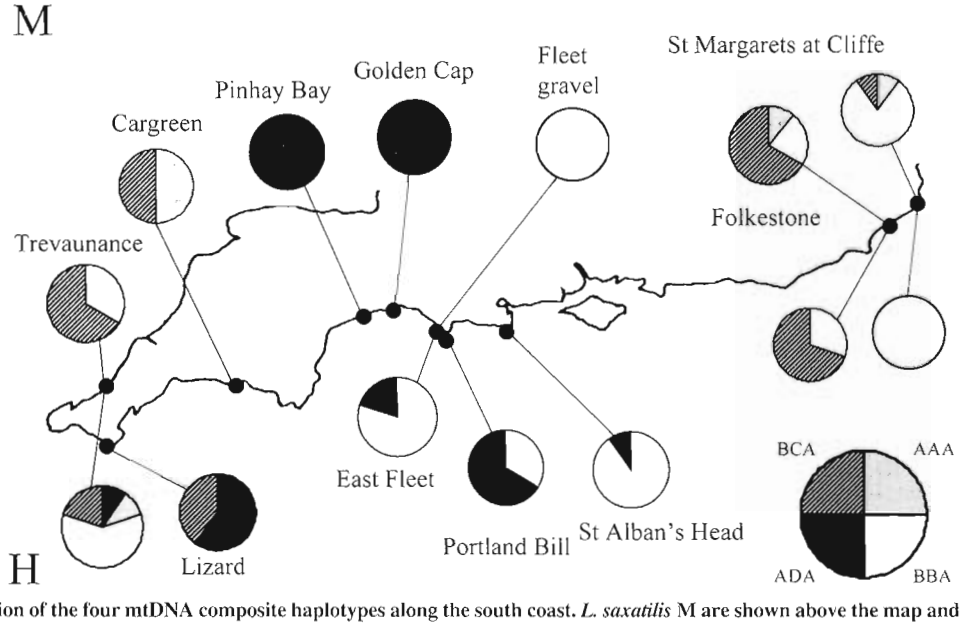

Figure 6. Distribution of the four mtDNA composite haplotypes along the south coast. *L. saxatilis* M are shown above the map and *L. saxatilis* H below.

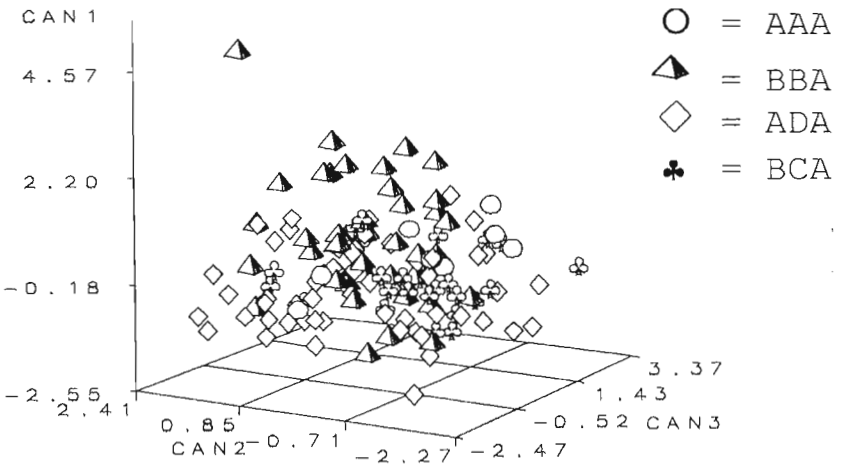

Figure 7. Plotting of haplotype onto canonical variate plot of shape measurements on an individual basis.

that clearer genetic differences have not been detected. However, the rough periwinkle group is itself young (Reid 1996) and sorting of mtDNA in recognized species has not gone to completion (Wilding et al. 2000a). Therefore the pattern within currently recognized species is likely to be complex, as noted here.

Is there a general framework for these observations? Apparently an aspect of the polymorphism of *L. saxatilis* (and perhaps of direct-developing intertidal snails in general) is the repeated appearance of similar phenotypes in different habitat regions of the shore, in response to similar selective pressures imposed by these habitats. Thus in the Galician region of Spain there are found a high-shore, ridged, banded and large morph (RB), and a low-shore, smooth, unbanded and small morph (SU) of *L. saxatilis*. These morphs show a variety of differences, considered to be at least partly genetically controlled, and are partially reproductively isolated (Johannesson et al. 1993). The likely selective factors are considered to be for small and slow growing snails in the lower shore, with larger and faster growing snails in the upper shore (Johannesson, Rolán-Alvarez and Erlandsson 1997). In the British Isles there are completely different morphs, referred to here as H and M, and it may be supposed that an important selective pressure is that of crab predation in the lower shore. Thus, *L. saxatilis* M closely resembles the common morph of *L. compressa* Jeffreys a low shore rough periwinkle, while *L. saxatilis* H is very like the typically higher shore *L. arcana* Hannaford Ellis. These H and M forms are like those in Sweden referred to as E and S (Janson 1982), but while in Britain there is evidence for partial reproductive isolation between H and M (Hull et al. 1996, Hull 1998, Pickles & Grahame 1999), this has not been reported previously for the Swedish animals (Erlandsson & Rolán-Alvarez 1998). Our observation of some difference in mtDNA haplotype frequency between them suggests that the E and S situation should be examined further.

The repeated nature of such phenotypic differences over large spatial scales (~1,000 km) together with the evidence of nascent reproductive barriers, yet superimposed on this undoubted evidence of gene flow between H and M (and E/S in Sweden, RB/SU in Spain), suggests analogy with the parallel speciation scenario proposed by Schluter and Nagel (1995) for sticklebacks. Here the proposed scale becomes important: it may be easier to envisage gene flow in *Littorina* populations along the British coast than between stickleback populations in isolated lakes, but gene flow between snail populations in Spain and Britain may well be very small. In addition the parallel speciation scenario may be applicable even over smaller scales (within Britain, within Galicia). Evidence to date from Galician *L. saxatilis* (Johannesson et al. 1983) and H and M around Britain for neutral loci (Wilding et al. in press) supports the interpretation that populations are more closely related at a site than between sites and yet display the same pattern of morphological differentiation.

The application of additional markers with replicated samples will aid in uncovering the genetic basis underlying *L. saxatilis* H and M. Further repeated sampling, as for that implemented at Galloway, is also needed to test if the patterns at Mumbles and Ursholmen, Sweden are robust to repeated sampling or simply an artifact brought about by the small sample sizes. It is clear that much more needs to be done on the biogoegraphy, behavior, and

TABLE 3.

Discriminant analysis with cross validation on canonical variance with haplotypes as predictors.

	Original haplotype group			
Destination haplotype group	AAA	BBA	ADA	BCA
AAA	62.50%	12.50%	12.50%	12.50%
BBA	11.70%	41.70%	23.30%	23.30%
ADA	22.00%	22.00%	41.50%	14.70%
BCA	38.50%	19.20%	25.90%	30.80%

TABLE 4.

Nested analysis of molecular variance (AMOVA) of RFLP variation in Littorina.

Source of variation	% of total
Group = shape zones	
among groups	6.78
among populations within groups	52.31
within populations	40.90
Group = 'H' and 'M'	
among groups	-6.70
among populations within groups	63.48
within populations	43.22

See Table 1 for details of placement of samples into particular groupings.

genetic consitution of the forms of *L. saxatilis* as an interesting example of speciation in progress.

ACKNOWLEDGMENTS

This research was supported by the MAST-3 program of the European Commission under contract number MAS3-CT95-0042 (AMBIOS). CSW is grateful for a grant from the Genetical Society of Great Britain toward attendance at the 6th International Littorinid conference. We wish to thank David Reid of the Natural History Museum for the samples of Swedish *Littorina saxatilis*. Paul Chippindale and one anonymous referee provided helpful comments on this manuscript.

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