# Generic Variation in Sympatric Sibling Species of *Littorina*

BY

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## INTRODUCTION

A NUMBER OF STUDIES on marine invertebrates have correlated spatial heterogeneity in allele frequencies at certain enzyme gene loci with the dispersal ability of organisms (WIUM-ANDERSEN, 1970; SCHOPF & GOOCH, 1971; GOOCH *et al.*, 1972; SNYDER & GOOCH, 1973; BERGER, 1973, 1977; GAINES *et al.*, 1974; WILKINS *et al.*, 1978). In general, these studies have indicated that the difference in allele frequencies between geographically separated populations is greater in species which lack a planktonic stage in the life cycle than in those with planktonic eggs or larvae, or both. Site-specific, or region-specific, alleles are also more common in the former than in the latter. These conclusions have, to a large extent, been drawn from studies on littorinids or from comparisons of other taxonomically unrelated species with littorinids. At the loci encoding esterases (BERGER, 1973) phosphoglucose isomerase and phosphoglucomutase (WILKINS *et al.*, 1978) intersite allele frequency variations are greater and site specific alleles more common in *Littorina saxatilis* (Olivi, 1792) which is ovoviviparous than in *L. littorea* (Linnaeus, 1758) which has planktonic eggs and larvae. *Littorina obtusata* (= *L. littoralis*) which has benthic eggs is intermediate between these two.

Results such as these on littorinids may be influenced by the taxonomic status of the species investigated. If, for example, *Littorina saxatilis* were composed of two or more distinct species with characteristic allele frequencies, the observed geographic variation might reflect differing distributions or proportions of these species at the various sites. The most recent evidence (HELLER, 1975) indicates that in the European area at least, "*L. saxatilis*" may comprise four separate, non-interbreeding species viz. *L. rudis* (Maton, 1797), *L. nigrolineata* Gray, 1839, *L. neglecta* Bean and *L. patula* Jeffreys. Likewise, two distinct species comprise the "*L. obtusata* (= *littoralis*)" complex viz. *L. obtusata* (Linnaeus) and *L. mariae* Sacchi & Rast. (SACCHI & RASTELLI, 1967).

We have examined over 3 000 winkles, separated morphologically into these species, from 9 sites in Ireland and one site in France. We present here the results of the analysis of genic variability at the phosphoglucose isomerase and phosphoglucomutase loci in these samples. The results indicate that genic variability does correlate with dispersal capability for the species *Littorina rudis*, *L. obtusata* and *L. littorea*, but extrapolation from these to other species is not warranted. The significantly greater degree of genic variability in *L. rudis* is unexpected when the potential for inbreeding in this ovoviviparous species is considered.

## MATERIALS AND METHODS

Samples were collected from rocky shores at 6 sites (Silverstrand, Barna, Spiddal, Carna, Doolin, Aranmore island) in or near Galway Bay on the west coast of Ireland, and from one site near each of the localities Cork (south coast), Carnsore (southeast coast), Dublin (east coast) and Brest (west coast of Brittany, France), as indicated in Table 1.

### Table 1

Numbers of individuals of the six species of winkles analyzed from the various sites. SIL., Silverstrand; BAR, Barna; SPI, Spideal; CAR, Carna; ARA, Aran Island; DOO, Doolin; COR, Cork; CAN, Carnsore; DUB, Dublin; BRE, Brest.

| Site | *Littorina littorea* | *Littorina nigro.* | *Littorina neglecta* | *Littorina rudis* | *Littorina obtusata* | *Littorina mariae* |
|---|---|---|---|---|---|---|
| SIL. | 56 | 77 | 90 | 60 | 72 | 101 |
| BAR. | 90 | 118 | 66 | 127 | 157 | 79 |
| SPI. | 146 | – | – | – | 171 | – |
| CAR. | 60 | – | 54 | – | 77 | – |
| ARA. | 45 | – | – | – | 86 | – |
| DOO. | 55 | 52 | – | 75 | – | – |
| COR. | 58 | 61 | – | 25 | 96 | – |
| CAN. | 73 | – | 71 | 79 | 83 | – |
| DUB. | 90 | – | – | 60 | 160 | – |
| BRE. | 75 | – | – | 74 | 78 | – |
| TOTALS | 748 | 308 | 281 | 500 | 980 | 180 |

Not all individuals from each site were analyzed for both enzymes; the figures in Table 1 are the maximum number analyzed at one or other gene locus from each site. Species were identified and separated using the criteria described by HELLER (1975) for the *Littorina saxatilis* complex and those of SACCHI & RASTELLI (1967) for the *L. obtusata* complex. We were unable to identify winkles of the *L. patula* type in any of the samples. Individuals were maintained alive in sea-water aquaria until analyzed by the usual electrophoretic technique (WILKINS, 1977).

Allele frequencies in the different populations or species, or both, were compared by $X^2$ analysis of 2xj tables of observed allele numbers, with one or two degrees of freedom as appropriate.

## RESULTS

The electrophoretic patterns observed were consistent with those previously reported (WILKINS *et al.*, 1978). Phosphoglucose isomerase (Pgi) patterns indicated a dimeric enzyme encoded at a single locus in all the species, with 2, 3 or 4 alleles expressed in each species. Mobilities of the F, F', S, and S' isozymes were similar in all species except for *Littorina littorea* in which all Pgi isozymes migrated more anodally than in the other species. Phosphoglucomutase patterns indicated that this enzyme is monomeric, encoded at a single locus with 1, 2 or 3 alleles in each species. Isozyme mobilities were similar in all species except *L. littorea* in which all isozymes migrated more slowly than in the other species.

Table 2 summarizes the results obtained for all 6 species at both loci. The 3 species of the *saxatilis* complex did, indeed, have characteristic allele frequency distributions: at both loci all 3 species differed significantly ($P < 0.001$), except for *Littorina rudis* and *L. neglecta* at the Pgi locus. These differences between the species were statistically significant ($P < 0.001$) at all localities where the species occurred together; *i. e.*, sympatric populations of the various species were significantly different from each other. Considering all localities, *Littorina nigrolineata* differed significantly from *L. rudis* and from *L. neglecta* at both loci in all possible between-site and inter-species comparisons. The difference between *L. rudis* and *L. neglecta* was statistically significant in all between-site comparisons at the Pgm locus, but only in 2 of 24 comparisons at the Pgi locus.

**Table 2**

Summary of genic variability at the Pgi and Pgm loci in six species of *Littorina*.

| | Phosphoglucose Isomerase | | | | | | Phosphoglucomutase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | $N_p$ | $N_t$ | pF | pS | pF' | pS' | $N_p$ | $N_t$ | pF | pF | pF' |
| *Littorina littorea* | 10 | 747 | 0.957 (0.0070) | 0.043 (0.0070) | — | — | 10 | 593 | 0.951 (0.0051) | 0.021 (0.0028) | 0.028 (0.0070) |
| Saxatilis complex | | | | | | | | | | | |
| *Littorina nigrolineata* | 4 | 308 | 0.955 (0.0125) | 0.045 (0.0120) | — | — | 4 | 299 | 0.840 (0.0560) | 0.141 (0.0595) | 0.026 (0.0145) |
| *Littorina neglecta* | 4 | 281 | 0.682 (0.0225) | 0.280 (0.0080) | 0.048 (0.0170) | — | 3 | 216 | 0.412 (0.1062) | 0.541 (0.0895) | 0.048 (0.0173) |
| *Littorina rudis* | 6 | 416 | 0.708 (0.0245) | 0.257 (0.0253) | 0.026 (0.0061) | 0.015 (0.0012) | 7 | 473 | 0.539 (0.0227) | 0.322 (0.0442) | 0.015 (0.0299) |
| Obtusata complex | | | | | | | | | | | |
| *Littorina obtusata* | 8 | 891 | .714 (0.0159) | 0.235 (0.0134) | 0.050 (0.0088) | — | 8 | 691 | 0.327 (0.0251) | 0.673 (0.0255) | 0.006 (0.0) |
| *Littorina mariae* | 2 | 180 | 0.585 (0.0092) | 0.412 (0.0057) | 0.006 (0.0071) | — | 2 | 180 | 1.00 | — | — |

$N_p$ = number of populations sampled.

$N_t$ = number of individuals analyzed.

pF, pF', pS, pS' = mean frequencies of the various alleles. The number in parentheses under each allele frequency is the standard error of the mean frequency (S.E.M.).

*Littorina obtusata* differed significantly from *L. mariae* at both loci. This difference between them was statistically significant ($P < 0.001$) at each of the localities (Silverstrand and Barna) from which both species were collected, and the differences were also significant in all comparisons of the 2 species considered over all sites.

Interpopulation variation in allele frequency was less in *Littorina littorea* than in most of the other species. This is reflected in the generally lower standard error of the mean given with each allele frequency in Table 2. Interspecific comparisons of allele frequency variance are more meaningful when restricted to sites common to all the species sampled. Thus, when *L. littorea, L. obtusata* and *L. rudis* representing species which have planktonic eggs, benthic eggs and internally brooded eggs, respectively, were compared over the 5 sites from which samples of all 3 species were collected (Table 3), the standard error of the mean frequencies increased from *L. littorea* through *L. obtusata* to *L. rudis*. Although the lower variability in *L. littorea* may be attributable to the more extreme values of the allele frequencies in the species (variance decreases as allele frequency approached 1 or 0), the variance of *L. obtusata* was consistently less than that of *L. rudis*, but their mean frequencies were of intermediate value and similar to each other.

At the population level, these differences in geographic variability between the species were most obvious. When populations of *Littorina littorea* were compared in all possible pairwise combinations, no statistically significant differences in allele frequency were observed in any comparison at either locus. In contrast, *L. obtusata* populations differed significantly in 8 out of 28 comparisons (29%) at the Pgi locus and in 14 out of 28 comparisons (50%) at the Pgm locus. *Littorina rudis* populations differed significantly in 2 out of 15 comparisons (13%) at the Pgi locus and in 18 out of 21 comparisons (86%) at the Pgm locus.

In Table 4 the observed heterozygosity is compared between the various species and their mode of reproduction is indicated. Heterozygosity levels are significantly lower in *L. littorea* and *L. nigrolineata* than in any of the other species, excepting *L. mariae* where a single allele is fixed at the Pgm locus.

## Table 3

Comparison of allele frequencies at the Pgi and Pgm loci in *Littorina littorea, Littorina obtusata and Littorina rudis* from the six sites at which all three species were collected. Site abbreviations as in Table 1.

| | Phosphoglucose isomerase | | | | | | Phosphoglucomutase | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *L. littorea* | | *L. obtusata* | | *L. rudis* | | *L. littorea* | | *L. obtusata* | | *L. rudis* | |
| Site | F | S | F | S | F | S | F | S | F | S | F | S |
| SIL. | 0.938 | 0.063 | 0.721 | 0.214 | 0.746 | 0.246 | 0.955 | 0.027 | 0.375 | 0.625 | 0.667 | 0.208 |
| BAR. | 0.967 | 0.033 | 0.732 | 0.194 | 0.702 | 0.250 | 0.972 | 0.011 | 0.349 | 0.651 | 0.406 | 0.370 |
| COR. | 0.931 | 0.069 | 0.690 | 0.288 | 0.740 | 0.220 | 0.956 | 0.018 | 0.432 | 0.568 | 0.391 | 0.457 |
| WEX. | 0.979 | 0.021 | – | – | 0.684 | 0.289 | 0.918 | 0.014 | 0.325 | 0.675 | 0.481 | 0.430 |
| DUB. | 0.944 | 0.056 | 0.728 | 0.241 | 0.600 | 0.358 | 0.938 | 0.025 | 0.228 | 0.772 | 0.707 | 0.195 |
| BRE. | 0.947 | 0.053 | 0.615 | 0.288 | 0.777 | 0.203 | 0.954 | 0.033 | 0.218 | 0.776 | 0.561 | 0.365 |
| MEAN | 0.951 | 0.049 | 0.697 | 0.245 | 0.708 | 0.261 | 0.949 | 0.021 | 0.321 | 0.679 | 0.536 | 0.338 |
| S.E.M. | 0.007 | 0.008 | 0.022 | 0.019 | 0.026 | 0.023 | 0.008 | 0.003 | 0.034 | 0.034 | 0.054 | 0.045 |

## DISCUSSION

The absence of significant gene exchange between *Littorina rudis* and *L. nigrolineata* which is indicated by their different allele frequencies at all localities, but especially at those (Silverstrand, Barna, Doolin and Cork) where sympatric populations were studied, strongly supports the proposal of Heller (1975) that these constitute 2 separate biological species. *Littorina nigrolineata* is also clearly different at both loci, even in sympatric populations, from

## Table 4

Mode of reproduction and degree of genetic variability in winkles.

| Species | Reproduction | | | Phosphoglucose isomerase | | | | Phosphoglucomutase | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mode | Egg | Larva | $N_t$ | $N_a$ | Het | p | $N_t$ | $N_a$ | Het | p |
| *Littorina littorea* | ovip. | planktonic | planktonic | 747 | 2 | 0.078 | 0.957 | 593 | 3 | 0.099 | 0.951 |
| *Littorina nigrolineata* | ovip. | benthic | non-planktonic[1] | 308 | 2 | 0.090 | 0.955 | 299 | 3 | 0.118 | 0.840 |
| *Littorina obtusata* | ovip. | benthic | crawling | 891 | 3 | 0.447 | 0.714 | 691 | 3 | 0.472 | 0.673 |
| *Littorina mariae* | ovip. | benthic | crawling | 180 | 3 | 0.508 | 0.585 | 180 | 1 | 0.00 | 1.00 |
| *Littorina rudis* | ovovivip. | brooded | crawling | 416 | 4 | 0.395 | 0.708 | 473 | 3 | 0.582 | 0.539 |
| *Littorina neglecta* | ovovivip. | brooded | crawling | 281 | 3 | 0.413 | 0.682 | 216 | 3 | 0.495 | 0.541 |

[1]Oviparity, with benthic eggs, was described for forms now classified (Heller, 1975) as *L. nigrolineata* as early as 1947, in a much neglected report by Seshappa (1947). Heller (1975) indicates that the larvae are non-planktonic but we are not aware of any published record of this fact. Indeed, the resemblance of *L. nigrolineata* to *L. littorea* in its reduced heterozygosity would be easily explained if *L. nigrolineata* proves to have planktonic larvae.

$N_t$ = number of individuals analyzed.

$N_a$ = number of alleles detected.

Het = proportion of heterozygotes observed.

p = mean frequency of the commonest allele.

*L. neglecta*. However, it has been reported that copulation by male *L. rudis* with individuals other than conspecific females may be as high as 50%, and male *L. nigrolineata* exhibit similar non-selectivity in copulatory behavior (Raffaelli, 1978). For each species, "wrong" copulations comprise those made with conspecific males and with males and females of the other species. The difference observed here between the allele frequencies in sympatric populations of these species indicate that those "wrong" copulations which involve females are not reproductively successful. On this genetic evidence, together with morphological and other data (Heller, 1975; Sacchi *et al.*, 1977), *L. nigrolineata* can be regarded as a good species reproductively isolated from both *L. rudis* and *L. neglecta*.

*Littorina rudis* and *L. neglecta* differ only at the Pgm locus. Since this difference is statistically significant ($P < 0.001$) over all localities, and especially in sympatric populations (Silverstrand, Barna, Carnsore), it indicates that uninterrupted gene flow does not occur between these species either. *Littorina obtusata* and *L. mariae* differ at both loci in sympatric populations and these genetic data support the other evidence (Sacchi & Rastelli, 1966; Goodwin & Fish, 1977) used to distinguish these species. The absence of hybridization between *L. littorea* and any of the other species is unequivocal since *L. littorea* shared no allele at either locus with any of the other species and no individual was observed to possess a combination of any *L. littorea* allele with one from another species. In view of the widespread citation of these littorinid species, especially "*L. saxatilis*," in studies on the ecology, physiology and behavior of littoral organisms, a detailed survey of the distribution of the various species is warranted, particularly on the east coast of North America where the presence of *L. nigrolineata* Gray, *L. neglecta* Bean and *L. mariae* Sacchi & Rastelli, 1966 has yet to be determined.

The observations made here on geographic variability and its correlation with dispersal capability confirm the earlier results of Berger (1973) and Wilkins *et al.* (1978) for the species *Littorina rudis, L. obtusata* and *L. littorea*. In neither of these earlier papers were the species within the *saxatilis* and *obtusata* (*littoralis*) complexes precisely identified. While supporting the earlier results for these species, the extended data presented here indicate that the correlation of genic variability with dispersal capability is not simple, and extrapolation from these to other, even other littorinid, species is not justified. Compare, for example, the Pgm allele variances of oviparous *L. nigrolineata* and ovoviviparous *L. rudis*, or the Pgi allele variances of oviparous *L. obtusata* and ovoviviparous *L. neglecta* (Table 2).

We wish to draw attention to one final interesting feature of the data: this is the high absolute level of genic heterozygosity in the ovoviviparous species (*Littorina rudis* and *L. neglecta*, Table 4). This observation is not

confined to the two loci studied here. High overall heterozygosity was also observed by BERGER (1978) at other loci in *L. saxatilis* (precise species not indicated, but probably *L. rudis*) from Roscoff and from Cape Cod. In all studies involving littorinids, the ovoviviparous *L. saxatilis* has consistently exhibited significant levels of genic heterozygosity, especially when contrasted with *L. littorea*. In ovoviviparous littorinids the fertilized ova are retained within the brood pouch of the female until development is complete, when a crawling juvenile emerges. There is no active dispersal phase in the life cycle and the juveniles live largely within the range of their own parents. Under such circumstances, effective population sizes are small and inbreeding is likely to be common. Since inbreeding and random genetic drift in populations of small effective size both act to increase homozygosity, the observed high heterozygosity is most unexpected. The possibility cannot be excluded that individuals of a newly recognised species, *Littorina arcana* (HANNAFORD ELLIS, 1978) may have been included among those identified here as *L. rudis*. *Littorina arcana* resembles *L. rudis* in shell and penial characters, but differs in reproducing oviparously (HANNAFORD ELLIS, *op. cit.*). If our *L. rudis* samples are indeed a mixture of these two species (although we have no reason to suspect they are), this could explain the high genic variability observed. This possibility needs further investigation. However, no such problem exists in the case of *L. neglecta:* in this ovoviviparous species at least, natural selection must favour heterozygotes strongly in order to maintain the actual levels of heterozygosity observed.

## SUMMARY

Sympatric populations of the sibling species *Littorina rudis* (Maton), *L. neglecta* Bean and *L. nigrolineata* Gray, all until recently regarded as subspecies or varieties of *L. saxatilis* Olivi, have different allele frequencies at the gene loci encoding phosphoglucose isomerase and phosphoglucomutase. Sympatric populations of *L. obtusata* (Linnaeus) and *L. mariae* Sacchi & Rastelli also differ significantly from each other at these loci. Geographic variability at these loci is greatest in *L. rudis* and least in *L. littorea*, but the correlation of genic variability with dispersal capability is less obvious than previously reported. The unexpectedly high absolute level of heterozygosity in ovoviviparous *L. rudis* and *L. neglecta* indicates that natural selection maintains variability in these species.

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