# ALLOZYME SIMILARITY OF ATLANTIC AND PACIFIC SPECIES OF *LITTORINA* (GASTROPODA: LITTORINIDAE)

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## ABSTRACT

Four *Littorina* species from the Pacific (*L. sitkana, L. brevicula, L. mandshurica, L. squalida*) and four from the Atlantic (*L. mariae, L. obtusata, L. saxatilis* and *L. littorea*) were compared electrophoretically using 17 loci.

Analysis of genetic identities showed that there were three groups. The first group comprises *L. sitkana, L. mariae, L. obtusata* and *L. saxatilis*. The second group consists of *L. brevicula* and *L. mandshurica* and the third one includes *L. squalida* and *L. littorea*. Our results support Reid's hypothesis of the origin of Atlantic littorines from two Pacific ancestors.

## INTRODUCTION

One of the main aims of systematics is to determine the phylogenetic sequences leading to modern forms. Electrophoretic data are now widely used in systematic studies (Buth, 1984). To be of value for systematics the character should meet two important criteria. First, the observed variation should have a genetic basis. Second, each taxonomic character should be independent of all other characters used. Electrophoretic data meet both these criteria (Richardson *et al.*, 1986). Moreover, the rate of nucleotide and amino acid substitutions are thought to be relatively constant. Such constancy of change permits the use of electrophoretic data for systematic studies (Nei, 1987).

Species of the genus *Littorina* have been a difficult group for taxonomists. Electrophoretic data have helped to confirm the status of some species, e.g. those of the '*saxatilis*', '*obtusata*' and '*scutulata*' species complexes (Ward & Warwick, 1980; Mastro *et al.*, 1982; Janson, 1985; Janson & Ward, 1985; Ward & Janson, 1985; Ward, 1990; Johannesson & Johannesson, 1990; Knight & Ward, 1991). Many studies have been devoted to the investigation of population

structure in littorines (Ward & Warwick, 1980; Janson & Ward, 1984; Janson, 1987; Knight *et al.*, 1987; Knight & Ward, 1991). However, there have been few examples of the use of electrophoretic data for examining phylogenetic relationships in the genus *Littorina* (Ward, 1990 and references therein). These few cases have compared only Atlantic species and there have been no attempts to compare Atlantic and Pacific species genetically. The difficulty of such investigation is that simultaneous electrophoresis of as many species as possible is needed.

Genetic comparison of Atlantic and Pacific littorines is especially interesting now, because recently Reid (1990a) proposed a cladogram of the genus *Littorina* based on morphological characters. According to Reid (1990b) the northern Atlantic species originated from at least two immigrants from the Pacific. Atlantic *L. littorea* evolved from an ancestor common with Pacific *L. squalida*, while the other Atlantic *Littorina* came from an ancestor common with Pacific *L. sitkana*. It is of interest to know whether electrophoretic data support the hypothesis. If the hypothesis is true, it can be predicted that *L. squalida* and *L. littorea* should be more genetically similar to each other than to other littorines and *L. sitkana* should be more similar to the remaining Atlantic littorines.

In this paper a comparative electrophoretic analysis of four Pacific species (*L. sitkana* Philippi, *L. brevicula* (Philippi), *L. mandshurica* Schrenck, *L. squalida* Broderip & Sowerby) and four northern Atlantic species (*L. saxatilis* (Olivi), *L. mariae* Sacchi & Rastelli, *L. obtusata* (L.), *L. littorea* (L.)) was conducted using 17 enzyme loci.

## MATERIAL AND METHODS

Samples of all the species except *L. mandshurica* and *L. squalida* were collected from two sites. Atlantic

species were from both the White and Barents Seas. Pacific species were from two points in Peter the Great Bay, Sea of Japan. *L. mandshurica* and *L. squalida* were from Vostok Bay only.

Animals were kept alive until electrophoresis (which was done at the Vostok station, Institute of Marine Biology). Horizontal electrophoresis was carried out using 13% starch gels as described by Zaslavskaya (1989). Altogether, 26 enzymes and 31 loci were screened. However, some enzymes were not investigated (or were not revealed) in some samples or species. In all 14 samples 15 loci coding for 13 enzymes were investigated. All of the eight species were studied for 17 enzyme loci (Table 1).

To process the data, the BIOSYS (Swofford & Selander, 1981), NTSYS (Rohlf, 1988) and PHYLIP (Felsenstein, 1990) packages were used.

## RESULTS

A dendrogram for the 14 populations studied is given in Fig. 1. This dendrogram was produced from estimates of genetic distances (Nei, 1972) over 15 loci by UPGMA (unweighted pair-group method) using NTSYS. Because intraspecific differences were much smaller than interspecific differences, we will present data on pooled samples for each species.

Allele frequencies at each of 17 loci for the

eight species are given in Table 2. Estimates of genetic similarity and distance over all loci between all pairs of species are given in Table 3.

Figures 2 and 3 show the dendrograms of genetic relationships among the eight species based on data from Table 3. One of the dendrograms (Fig. 2) was constructed by UPGMA, which gives rooted trees, while the other was constructed by the unrooted Fitch-Margoliash method. Figure 4 is a dendrogram constructed by the Distance Wagner procedure based on Roger's distance. Each of these methods is based on different assumptions. The UPGMA method would correctly reflect phylogeny if the molecular clock hypothesis applies. The other two methods (Fitch-Margoliash and Distance Wagner procedure) are thought to give the correct phylogeny even if different rates of evolution took place in separate branches (Richardson et al., 1986).

## DISCUSSION

From each of the dendrograms (Figs 2, 3) we can readily distinguish two groups. The first group includes *L. saxatilis*, *L. mariae*, *L.*

**Table 1.** Enzymes assayed in eight species of *Littorina*.

| Enzyme | E. C. no. | Locus | Enzyme structure | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | sx | mr | ob | li | si | sq | br | mn |
| Alanopine dehydrogenase | 1.5.1.17 | Aldh | M | M | M | – | – | M | M | M |
| Esterase D | 3.1.1.* | Est D | D | – | – | – | D | – | – | – |
| Esterase X | 3.1.1.1 | Est X | M | – | M | M | M | – | M | – |
| Glutamate oxaloacetate transaminase | 2.6.1.1 | Got-1 | D | – | – | – | – | – | D | – |
| Glutathione reductase | 1.6.4.2 | Gr | – | – | – | – | – | – | – | – |
| Inorganic pyrophosphatase | 3.6.1.1 | Ipp | – | D | – | D | D | D | D | D |
| Isocitrate dehydrogenase | 1.1.1.42 | Idh | D | D | D | – | D | D | D | – |
| Malate dehydrogenase | 1.1.1.37 | Mdh-1 | – | – | – | – | – | – | – | – |
| | | Mdh-2 | – | – | D | – | – | – | – | – |
| Peptidase | 3.4.*.* | Pep-1 | M | M | M | – | – | – | – | – |
| (substrate used was leucyl-glycyl) | | Pep-2 | D | D | D | D | D | D | D | D |
| 6-Phosphogluconate dehydrogenase | 1.1.1.44 | 6-Pgd | – | – | D | D | D | – | D | – |
| Phosphoglucose isomerase | 5.3.1.9 | Pgi | D | D | D | D | D | D | D | D |
| Phosphoglucomutase | 2.7.5.1 | Pgm-1 | M | M | M | – | – | M | M | M |
| | | Pgm-2 | M | M | – | – | M | – | M | M |
| Sorbitol dehydrogenase | 1.1.1.14 | Sdh | – | D | D | – | – | – | D | – |
| Superoxide dismutase | 1.15.1.1 | Sod | D | D | D | – | – | – | – | – |

Enzyme structure: D, enzyme is dimer; M, monomer; –, variation was not revealed.

*Littorina* species: sx, *L. saxatilis*; mr, *L. mariae*; ob, *L. obtusata*; li, *L. littorea*; si, *L. sitkana*; sq, *L. squalida*; br, *L. brevicula*; mn, *L. mandshurica*.
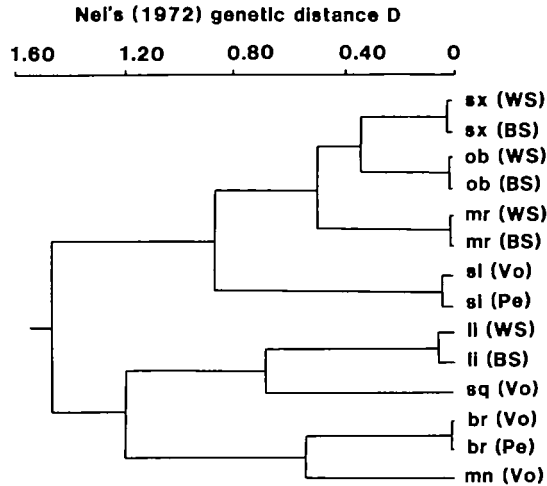
**Figure 1.** Genetic relationships between 14 populations of eight species of *Littorina* (UPGMA cluster analysis). Abbreviations: BS, Barents Sea; WS, White Sea; Pe and Vo, Peles Island and Vostok Bay, two populations in the Sea of Japan, species abbreviations as in Table 1.

*obtusata* and *L. sitkana*. Only when using the Distance Wagner procedure did the species of the *obtusata* complex, *L. obtusata* and *L. mariae*, cluster together (Fig. 4). Neither of the other two methods gave the correct picture, instead clustering *L. saxatilis* with *L. obtusata* (UPGMA, Fig. 2) or *L. saxatilis* with *L. mariae* (Fitch-Margoliash method, Fig. 3). Perhaps this discrepancy can be explained by large sampling error, because of the small number of loci used. The second group includes the *L. brevicula-L. mandshurica* cluster and the *L. squalida-L. littorea* cluster. The two groups recognized here on the basis of genetic similarity correspond respectively to the two subgenera *Neritrema* and *Littorina*, as used by Reid (1990a).

The genetic data support the hypothesis of Reid that the northern Atlantic species originated from two Pacific species: *L. littorea* in the Atlantic being the sister species of *L. squalida* in the Pacific, and the Atlantic species of *L. (Neritrema)* forming a monophyletic group with *L. sitkana* as a sister-taxon (Reid, 1990a, b). It is interesting to note that the genetic distance between *L. squalida* and *L. littorea* (D=0.689) and the mean genetic distance between *L. sitkana* and the three Atlantic species of *L. (Neritrema)* (D=0.811) are quite similar. If the molecular clock hypothesis holds, this implies that two Pacific species, from which the Atlantic species evolved, migrated to the northern Atlantic at approximately the same time. If we use Nei's

calibration of D (D of 1.0 represents $5 \times 10^6$ years) then our estimates of D will give 3.45–4.06 Ma, which corresponds to the time of opening of the Bering Strait, 3 to 4 Ma ago (Reid, 1990b and references therein).

The dendrogram (Fig. 2) also confirms the evolutionary sequence of the type of reproduction and development proposed by Reid (1990a): 1) pelagic egg capsules (*L. brevicula, L. mandshurica, L. squalida, L. littorea*), 2) egg capsules embedded in a benthic protective gelatinous mass (*L. sitkana*), 3) eggs with reduced capsules (or without capsules at all) in a benthic mass (*L. obtusata, L. mariae*), and finally 4) the egg mass being retained in the oviduct, resulting in ovoviviparity (*L. saxatilis*).

Some of our results do not agree with Reid's phylogeny. Thus, according to the electrophoretic data, *L. mandshurica* and *L. brevicula* are genetically closer to the species of *L. (Neritrema)* than are *L. squalida* and *L. littorea*. In contrast, Reid believed that *L. brevicula* and *L. mandshurica* diverged earlier from the littorines investigated here than did *L. squalida* and *L. littorea*. However, it should be remembered that genetic distance estimates are subject to large sampling error when the number of loci is small, as in our case.

Thus, the examination of genetic relationships of European and Asian littorines largely confirmed the phylogeny produced by comparative morphology.

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**Table 2.** Allele frequencies at 17 loci in eight species of *Littorina*.

| Locus | Species | | | | | | | |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|       | sx    | mr    | ob    | li    | si    | sq    | br    | mn    |
| **GPI** |     |       |       |       |       |       |       |       |
| (N)   | 95    | 77    | 96    | 71    | 65    | 50    | 48    | 44    |
| A     | .000  | .000  | .000  | .035  | .000  | .000  | .000  | .000  |
| B     | .000  | .000  | .000  | .965  | .000  | .000  | .000  | .000  |
| C     | .005  | .000  | .000  | .000  | .000  | 1.000 | .000  | .000  |
| D     | .000  | .000  | .000  | .000  | .269  | .000  | .000  | .057  |
| E     | .753  | .721  | .568  | .000  | .723  | .000  | .021  | .716  |
| F     | .242  | .279  | .432  | .000  | .008  | .000  | .958  | .227  |
| G     | .000  | .000  | .000  | .000  | .000  | .000  | .021  | .000  |
| **IPP** |     |       |       |       |       |       |       |       |
| (N)   | 92    | 73    | 94    | 67    | 60    | 42    | 45    | 43    |
| A     | .000  | .000  | .000  | .000  | .275  | .000  | .000  | .000  |
| B     | .000  | .000  | .000  | .000  | .608  | .000  | .000  | .000  |
| C     | .000  | .007  | .000  | .000  | .050  | .000  | .000  | .000  |
| D     | .000  | .986  | .000  | .239  | .017  | .238  | .089  | .070  |
| E     | .000  | .000  | .000  | .000  | .050  | .036  | .000  | .058  |
| F     | .000  | .007  | .000  | .761  | .000  | .726  | .856  | .837  |
| G     | .000  | .000  | .000  | .000  | .000  | .000  | .056  | .035  |
| H     | 1.000 | .000  | 1.000 | .000  | .000  | .000  | .000  | .000  |
| **PEP-1** |   |       |       |       |       |       |       |       |
| (N)   | 72    | 53    | 68    | 44    | 31    | 35    | 31    | 31    |
| A     | .000  | .000  | .000  | 1.000 | .000  | .000  | .000  | .000  |
| **PGM-2** |   |       |       |       |       |       |       |       |
| (N)   | 53    | 42    | 65    | 44    | 23    | 6     | 9     | 10    |
| A     | .000  | .000  | .000  | .000  | .000  | .000  | .556  | .000  |
| B     | .000  | .000  | .000  | .330  | .000  | .000  | .000  | .900  |
| C     | .000  | .000  | .000  | .091  | .087  | 1.000 | .444  | .000  |
| D     | .623  | .000  | 1.000 | .284  | .261  | .000  | .000  | .100  |
| E     | .000  | .000  | .000  | .000  | .196  | .000  | .000  | .000  |
| F     | .189  | .000  | .000  | .000  | .435  | .000  | .000  | .000  |
| G     | .000  | .000  | .000  | .295  | .000  | .000  | .000  | .000  |
| H     | .189  | .369  | .000  | .000  | .000  | .000  | .000  | .000  |
| I     | .000  | .631  | .000  | .000  | .022  | .000  | .000  | .000  |
| **6-PGD** |   |       |       |       |       |       |       |       |
| (N)   | 67    | 69    | 63    | 39    | 38    | 29    | 39    | 35    |
| A     | .000  | .000  | .040  | .000  | .000  | .000  | .000  | .000  |
| B     | 1.000 | 1.000 | .913  | .526  | .974  | .000  | 1.000 | .957  |
| C     | .000  | .000  | .000  | .103  | .026  | .000  | .000  | .029  |
| D     | .000  | .000  | .048  | .372  | .000  | 1.000 | .000  | .014  |
| **EST-D** |   |       |       |       |       |       |       |       |
| (N)   | 52    | 29    | 67    | 51    | 38    | 48    | 46    | 42    |
| A     | .000  | .000  | .000  | .000  | .000  | 1.000 | .000  | .000  |
| B     | .000  | .000  | .000  | 1.000 | .000  | .000  | .000  | .000  |
| C     | .990  | 1.000 | 1.000 | .000  | .684  | .000  | 1.000 | 1.000 |
| D     | .010  | .000  | .000  | .000  | .316  | .000  | .000  | .000  |
| **GR** |    |       |       |       |       |       |       |       |
| (N)   | 58    | 45    | 59    | 30    | 34    | 31    | 34    | 28    |
| A     | .000  | .000  | .000  | 1.000 | .000  | 1.000 | .000  | .000  |
| B     | .000  | .000  | .000  | .000  | .000  | .000  | 1.000 | .000  |
| C     | .000  | .000  | .000  | .000  | .000  | .000  | .000  | 1.000 |
| D     | .000  | 1.000 | 1.000 | .000  | .000  | .000  | .000  | .000  |
| E     | .000  | .000  | .000  | .000  | 1.000 | .000  | .000  | .000  |
| F     | 1.000 | .000  | .000  | .000  | .000  | .000  | .000  | .000  |

**Table 2.** (*contd.*).

| Locus | sx | mr | ob | li | si | sq | br | mn |
|---|---|---|---|---|---|---|---|---|
| **ALDH** | | | | | | | | |
| (N) | 35 | 29 | 41 | 38 | 40 | 37 | 32 | 23 |
| A | .400 | .948 | .915 | .000 | .000 | .000 | .000 | .000 |
| B | .586 | .034 | .049 | .000 | .000 | .716 | .016 | .022 |
| C | .014 | .017 | .037 | 1.000 | .000 | .284 | .000 | .000 |
| D | .000 | .000 | .000 | .000 | 1.000 | .000 | .000 | .000 |
| E | .000 | .000 | .000 | .000 | .000 | .000 | .938 | .130 |
| F | .000 | .000 | .000 | .000 | .000 | .000 | .047 | .848 |
| **MDH-1** | | | | | | | | |
| (N) | 56 | 50 | 50 | 46 | 31 | 26 | 24 | 28 |
| A | 1.000 | 1.000 | 1.000 | .000 | .000 | .000 | .000 | .000 |
| B | .000 | .000 | .000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **MDH-2** | | | | | | | | |
| (N) | 56 | 50 | 50 | 46 | 31 | 26 | 24 | 28 |
| A | .000 | .000 | .030 | .000 | .000 | .000 | .000 | .000 |
| B | 1.000 | 1.000 | .870 | .054 | .000 | .000 | .000 | .000 |
| C | .000 | .000 | .100 | .946 | 1.000 | .885 | 1.000 | 1.000 |
| D | .000 | .000 | .000 | .000 | .000 | .115 | .000 | .000 |
| **GOT-1** | | | | | | | | |
| (N) | 71 | 57 | 61 | 54 | 48 | 36 | 32 | 27 |
| A | .092 | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| B | .000 | .000 | .000 | 1.000 | .000 | 1.000 | .000 | .000 |
| C | .000 | .000 | 1.000 | .000 | .000 | .000 | .000 | .000 |
| D | .000 | .000 | .000 | .000 | .000 | .000 | .969 | 1.000 |
| E | .901 | 1.000 | .000 | .000 | 1.000 | .000 | .031 | .000 |
| F | .007 | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| **IDH** | | | | | | | | |
| (N) | 64 | 52 | 56 | 35 | 13 | 25 | 14 | 6 |
| A | .078 | .038 | .170 | .000 | .000 | .000 | .000 | .000 |
| B | .000 | .010 | .009 | .000 | .000 | .000 | .000 | 1.000 |
| C | .867 | .952 | .777 | .000 | .962 | .000 | .286 | .000 |
| D | .000 | .000 | .000 | .000 | .000 | .980 | .000 | .000 |
| E | .000 | .000 | .000 | 1.000 | .000 | .020 | .000 | .000 |
| F | .055 | .000 | .045 | .000 | .038 | .000 | .714 | .000 |
| **SDH** | | | | | | | | |
| (N) | 35 | 27 | 31 | 25 | 17 | 16 | 12 | 8 |
| A | .000 | .019 | .000 | .000 | .000 | .000 | .000 | .000 |
| B | .000 | .944 | .984 | .000 | .000 | .000 | .750 | 1.000 |
| C | 1.000 | .037 | .016 | .000 | 1.000 | .000 | .250 | .000 |
| D | .000 | .000 | .000 | 1.000 | .000 | 1.000 | .000 | .000 |
| **SOD** | | | | | | | | |
| (N) | 52 | 37 | 51 | 19 | 14 | 11 | 21 | 6 |
| A | .144 | .095 | .029 | .000 | .000 | .000 | .000 | .000 |
| B | .856 | .905 | .971 | 1.000 | 1.000 | .000 | .000 | .000 |
| C | .000 | .000 | .000 | .000 | .000 | 1.000 | .000 | .000 |
| D | .000 | .000 | .000 | .000 | .000 | .000 | 1.000 | 1.000 |
| **EST-X** | | | | | | | | |
| (N) | 70 | 53 | 69 | 40 | 41 | 40 | 38 | 14 |
| A | .000 | 1.000 | .022 | .000 | .000 | .000 | .000 | .000 |
| B | .129 | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| C | .850 | .000 | .935 | 1.000 | .951 | 1.000 | .461 | 1.000 |
| D | .021 | .000 | .043 | .000 | .049 | .000 | .000 | .000 |
| E | .000 | .000 | .000 | .000 | .000 | .000 | .539 | .000 |

N, number of individuals assayed. Species abbreviations as in Table 1.

**Table 3.** Genetic identities (I, below diagonal) and distances (D, above diagonal) (Nei, 1972) between pairs of *Littorina* species.

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 *L. saxatilis* | – | .443 | .385 | 1.553 | .622 | 2.095 | 1.474 | 1.086 |
| 2 *L. mariae* | .642 | – | .489 | 2.129 | 1.055 | 3.713 | 1.457 | 1.196 |
| 3 *L. obtusata* | .681 | .613 | – | 1.445 | .756 | 1.658 | 1.312 | .969 |
| 4 *L. littorea* | .212 | .119 | .236 | – | 1.060 | .688 | 1.367 | 1.103 |
| 5 *L. sitkana* | .537 | .348 | .470 | .346 | – | 1.210 | 1.099 | .912 |
| 6 *L. squalida* | .123 | .024 | .191 | .503 | .298 | – | 1.446 | 1.322 |
| 7 *L. brevicula* | .229 | .233 | .269 | .255 | .333 | .235 | – | .573 |
| 8 *L. mandshurica* | .338 | .302 | .379 | .332 | .402 | .267 | .564 | – |
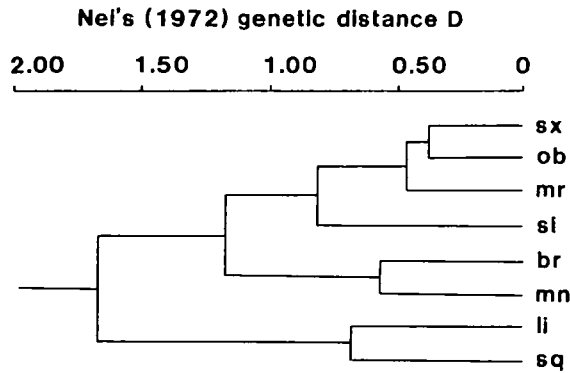


**Figure 2.** UPGMA tree of genetic relationships among eight species of *Littorina*. Species abbreviations as in Table 1.
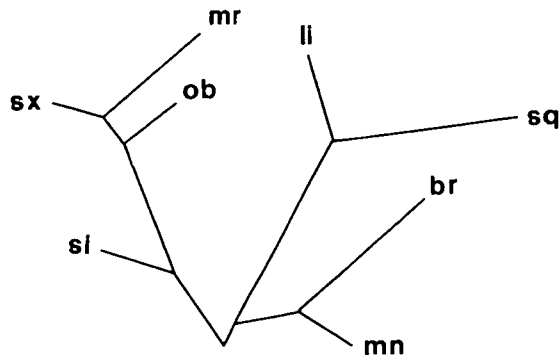


**Figure 3.** Relationships between eight species of *Littorina* (Fitch-Margoliash method). Species abbreviation as in Table 1.

## Postscript

Since this work was carried out, additional living specimens of *Littorina* have been obtained from southwestern Wales. In a separate study, A.N. Tatarenkov repeated the comparison of Atlantic and Pacific species, using the same four Pacific species, but *L. saxatilis*, *L. arcana* Hannaford Ellis and *L. nigrolineata* Gray from Wales. Thirteen loci were screened, only seven of which were among those examined in the earlier study (Table 1). The results obtained (Table 4, Fig. 5)
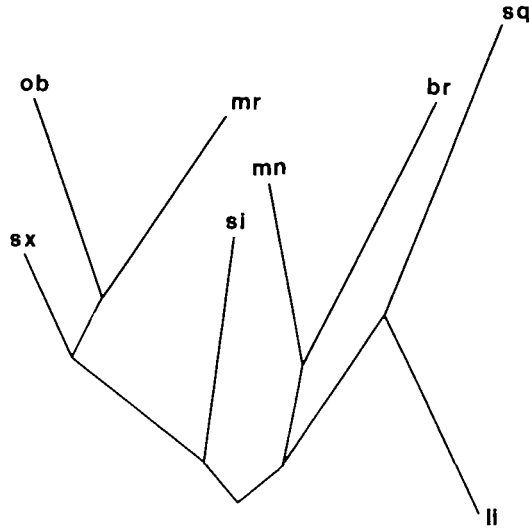
**Figure 4.** Relationships between eight species of *Littorina* (Distance Wagner procedure). Species abbreviations as in Table 1.

**Table 4.** Estimates of genetic identities (above diagonal) and standard genetic distances (below diagonal) in *Littorina* species from Peter the Great Bay (1–4) and from southwestern Wales (5–7) (data from A. N. Tatarenkov).

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 *L. squalida* | – | 0.204 | 0.182 | 0.271 | 0.114 | 0.085 | 0.085 |
| 2 *L. mandshurica* | 1.588 | – | 0.566 | 0.387 | 0.225 | 0.277 | 0.222 |
| 3 *L. brevicula* | 1.703 | 0.569 | – | 0.259 | 0.189 | 0.202 | 0.183 |
| 4 *L. sitkana* | 1.305 | 0.948 | 1.350 | – | 0.377 | 0.388 | 0.314 |
| 5 *L. saxatilis* | 2.169 | 1.490 | 1.668 | 0.974 | – | 0.938 | 0.740 |
| 6 *L. arcana* | 2.466 | 1.284 | 1.598 | 0.946 | 0.064 | – | 0.793 |
| 7 *L. nigrolineata* | 2.460 | 1.503 | 1.701 | 1.158 | 0.302 | 0.232 | – |

### Nei's (1972) genetic distance D
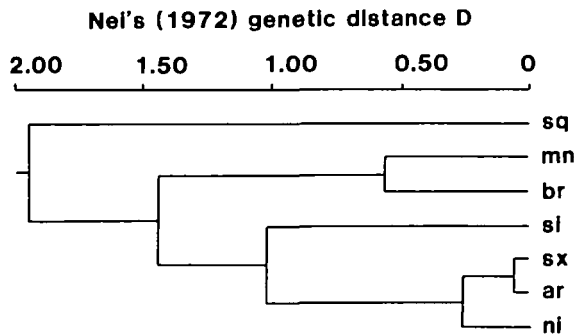


**Figure 5.** UPGMA tree of genetic relationships among seven species of *Littorina*. Species abbreviations as in Table 1 and ar, *L. arcana*; ni, *L. nigrolineata* (data from A.N. Tatarenkov).

were similar to those reported above, supporting Reid's (1990a) hypothesis of the origin of the *L. saxatilis* group from a common ancestor with *L. sitkana*. The finding of the earlier study that *L. brevicula* and *L. mandshurica* diverged from the species of *L. (Neritrema)* more recently than *L. squalida* was confirmed, in disagreement with the phylogeny derived from comparative morphological data by Reid (1990a). The inferred relationships among the three species of the *L. saxatilis* group were as reported by Ward (1990). The genetic distances between pairs of species were similar to those for the same pairs reported in the paper above, and by Ward (1990), except for the distance between *L. saxatilis* and *L. nigrolineata*, which at 0.302 is greater (though not significantly so) than the range of 0.042–0.237 (mean 0.106) given by Ward (1990).

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