Heredity **57** (1986) 233–241

Received 4 December 1985

Genetic analysis of ten polymorphic enzyme loci in *Littorina saxatilis* (Prosobranchia: Mollusca)

R. D. Ward,* T. Warwick† and A. J. Knight*

* Department of Human Sciences, Environmental Biology Unit, Loughborough University, Loughborough, Leicestershire, LE11 3TU, U.K.
† Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, U.K.

Electrophoretic surveys of natural populations have shown *Littorina saxatilis* to be variable at many enzyme loci. Laboratory breeding experiments, which involved the phenotypic examination of selected parental pairs and their offspring, confirmed, for ten loci, that this variation results from the segregation of codominant alleles. The loci studied were *Mpi*, *Pgi*, *Pgm*-1, *Pgm*-2, *Aat*-1, *Aat*-2, *Ap*-2, *Lap*-1, *Odh* and *Idh*-2. Certain pairs of loci showed evidence of weak linkage; no cases of strong linkage were detected. Crosses between male *L. saxatilis* and female *L. arcana* produced viable offspring with normal segregation of parental alleles. Attempts at the reciprocal cross have so far proved unsuccessful.

INTRODUCTION

In recent years, several papers have been published concerning the extent of genetic differentiation within and between populations of several species of the genus *Littorina* (*e.g.*, Snyder and Gooch, 1973; Berger, 1977; Ward and Warwick, 1980; Wilkins and O'Regan, 1980; Mastro *et al.*, 1982; Moyse *et al.*, 1982; Janson and Ward, 1984; Ward and Janson, 1985), a genus which comprises an important group of marine intertidal molluscs. In these studies, the genetic nature of the observed enzyme variation has been inferred, explicitly or implicitly, either by comparison with zymogram patterns of other animal species where formal breeding studies have been carried out or by comparison of phenotype distributions with the genotype distributions expected for genes in Hardy–Weinberg equilibria. Although such assumptions of Mendelian inheritance almost always prove well-founded, post-translational modification may sometimes produce enzyme variability that can be mis-scored as genetic variation. Hence, where possible, it is wise to confirm the validity of any genetic interpretations.

In the present paper, we present data from laboratory breeding experiments using *L. saxatilis* (= *L. rudis*) and 10 polymorphic loci which show that for these loci previously held genetic interpretations are indeed correct. The loci considered are mannose phosphate isomerase (*Mpi*), phosphoglucose isomerase (*Pgi*), phosphoglucomutases 1 and 2 (*Pgm*-1, *Pgm*-2), aspartate aminotransferases 1 and 2 (*Aat*-1, *Aat*-2), aminopeptidase-2 (*Ap*-2), leucine aminopeptidase-1 (*Lap*-1), octanol dehydrogenase (*Odh*) and isocitrate dehydrogenase-2 (*Idh*-2). We have also looked for evidence of linkage between many of these pairs of loci. Esterase variation is not considered in the present paper; here patterns are complex and, although clearly inherited, a final genetic model has yet to be formulated. Two of the successful crosses were between males of *L. saxatilis* and females of the closely related species *L. arcana*, and one cross was between a female *L. saxatilis tenebrosa* (= *L. tenebrosa*) and a male *L. saxatilis*.

Similar, although less extensive, breeding experiments have been carried out in a few other molluscan species such as the marine bivalves *Pecten maximus* (Beaumont *et al.*, 1986), *Mytilus edulis* (Hvilsom and Thiessen, 1984), and *Mercenaria mercenaria* (Adamkewicz *et al.*, 1984), and the terrestrial gastropod *Cepeae nemoralis* (Brussard and McCracken, 1974; Oxford, 1975; Johnson, 1979).

MATERIALS AND METHODS

Immature females, most of whom were taken from wild populations although some were laboratory

raised, were paired with wild-caught males, and the pairs isolated until live young were seen. Most crosses were between pairs of *Littorina saxatilis*, although some were between *L. arcana* females and *L. saxatilis* males. A full description of the breeding technique is given by Warwick (1983). When sufficient live young were seen, often after repeated matings, the parents were sacrificed and screened for electrophoretically detectable enzyme variation using the methods of Ward and Warwick (1980). If the presumed parental genotypes were such that screening of the offspring would provide useful information, individuals of the *F*1 generation were reared to a size of 2·5–4·0 mm and then electrophoresed. Each animal was homogenised in 25 μl of 0·2 M Tris-HCl, *p*H 8·0, providing sufficient extract for at least nine enzymes to be screened if desired. Locus and allele designations follow Ward and Warwick (1980) and Janson and Ward (1984).

RESULTS

The sources of animals used in the crosses are given in table 1. Many more crosses were established than indicated here, but some failed to produce offspring and in some others the parental genotypes proved insufficiently informative to merit screening of the progeny.

Littorina saxatilis is ovoviviparous, and laboratory crosses within this species resulted, as expected, in females producing live young, "crawlaways". *L. arcana* is oviparous, laying egg masses from which the crawlaways emerge, and it is interesting to note that in the laboratory, female *arcana* will mate with male *saxatilis* and produce egg masses and viable offspring. The progeny of two such crosses, numbers 1211 and 1625, are analysed here. The reciprocal cross has repeatedly failed to produce viable offspring. Crosses between pairs of *L. arcana*, although not described in the

Table 1 Sources and species of animals used in breeding experiments

Cross	Source	Species
872	♀ Loch Bee, South Uist, Scotland	"*tenebrosa*"
	♂ Oban a'Chlachain, North Uist, Scotland	*saxatilis*
1072	♀ Spiggie Bay, Shetland Isles	*saxatilis*
	♂ Aberlady (salting), East Lothian, Scotland	*saxatilis*
1080	♀ Lawrencetown beach, Nova Scotia, Canada	*saxatilis*
	♂ Aberlady (salting), East Lothian, Scotland	*saxatilis*
1111	♀ Spiggie Bay, Shetland Isles	*saxatilis*
	♂ Roxburgh Hotel (wall), Dunbar, Scotland	*saxatilis*
1114	♀ The Fleet at Abbottsbury, Dorset, England	*saxatilis*
	♂ Roxburgh Hotel (beach), Dunbar, Scotland	*saxatilis*
1135	♀ Oban a'Chlachain, North Uist, Scotland	*saxatilis*
	♂ Roxburgh Hotel (wall), Dunbar, Scotland	*saxatilis*
1211	♀ Roxburgh Hotel (wall), Dunbar, Scotland	*arcana*
	♂ Newford Island, St. Mary's, Scilly Isles, England	*saxatilis*
1625	♀ Roxburgh Hotel (wall), Dunbar, Scotland	*arcana*
	♂ Marine Villa, Dirleton (beach), Scotland	*saxatilis*
1686	♀, ♂ Seacliff (beach), North Berwick, Scotland	*saxatilis*
1698	♀ East Fleet at Moonfleet, Dorset, England	*saxatilis*
	♂ Dale Point, Pembrokeshire, Wales	*saxatilis*
1913	♀, ♂ laboratory raised from Davis Strait, Canada	*saxatilis*
1918	♀ Oban a'Chlachain, North Uist, Scotland	*saxatilis*
	♂ Aberlady (salting), East Lothian, Scotland	*saxatilis*
1926	♀ Davis Strait, Canada	*saxatilis*
	♂ Aberlady (salting), East Lothian, Scotland	*saxatilis*
1952	♀, ♂ Village Bay, Hirta, St. Kilda, Scotland	*saxatilis*
1926	♀ Davis Strait, Canada	*saxatilis*
	♂ Aberlady (salting), East Lothian, Scotland	*saxatilis*
1960	♀ Newford Island, St. Mary's Scilly Isles, England	*saxatilis*
	♂ Penzance (beach), Cornwall, England	*saxatilis*
2429	♀, ♂ Marine Villa, Dirleton (beach), Scotland	*saxatilis*
2451	♀ An *F*1 progeny from cross 1879	*saxatilis*
	♂ Village Bay, Hirta, St. Kilda, Scotland	*saxatilis*

present article, have been carried out in the laboratory, and viable egg masses are produced (Warwick, 1983, and unpublished observations). One cross (872) was between a female of the *tenebrosa* variety and a typical male *saxatilis*. Although it has been suggested that the form *tenebrosa* might be a distinct species within the *saxatilis* complex (*e.g.*, Fretter and Graham, 1980; Smith, 1982), recent morphological and genetical analyses indicate that it is best thought of as an ecotype of *L. saxatilis* (Janson and Ward, 1985).

Segregation of alleles at single loci

Breeding data are given in tables 2-6. In these tables, the identification number of the cross, parental genotypes, numbers and genotypes of offspring scored, fits to Mendelian expectations, and numbers of contaminants are given.

Contaminants are those individuals in the analysed progeny that had genotypes inconsistent with the parental genotypes at one or more of the loci scored in that progeny set. Progeny were normally scored for between five and seven loci, including the highly polymorphic esterases, and generally the contaminants had inconsistencies at two or more of these loci. Such aberrant individuals are much more readily explained by contamination than by high mutation rates or gene conversion events. The source of this contamination is not known for certain, but in the rearing of the progeny algal-covered pebbles are taken from the sea shore to act as a food source (Warwick, 1983). These pebbles were carefully inspected and any littorinids detected removed, but it is possible that a few juveniles were occasionally transferred from the shore into the culture dishes. This contamination rate is low: for example, in the *Mpi* crosses (table 2), a total of 924 juveniles were scored of which 12 were classified as contaminants (1·30 per cent) and 912 as true offspring. For other loci, the contamination rate was frequently below 1 per cent.

With respect to *Mpi*, 13 crosses were analysed (table 2). In previous population surveys (*e.g.*, Ward and Warwick, 1980; Ward and Janson, 1985; Janson, 1985), three alleles have been identified at the *Mpi* locus, *viz.*, Mpi^{120}, Mpi^{100} and Mpi^{75}, but it has been noted that the Mpi^{120} allele is a compound allele comprising two alleles of similar mobility that cannot always be unambiguously distinguished (Janson and Ward, 1984). In the parents and progeny examined in the breeding experiments, we were able to distinguish these two alleles, which are designated here Mpi^{120} and Mpi^{115}. All four alleles behave as true Mendelian genes, and progeny numbers of all crosses accorded with Mendelian expectations.

Data for the *Pgi* locus are presented in table 3. Again, we have previously noted that the allele we commonly term Pgi^{100} is a compound allele (Janson and Ward, 1984), and in cross 872 we were able to distinguish the two alleles, here designated Pgi^{100} and Pgi^{98}. Nine of ten crosses produce progeny in accord with Mendelian expectations, but cross 1918 showed a significant deviation ($P = 0{\cdot}027$). Here, observed (and expected) progeny numbers were 77 (92) Pgi^{100}/Pgi^{100} and 107 (92)

Table 2 Segregation of alleles at the *Mpi* locus

Cross number	Parental genotype		*N*	Progeny genotypes	χ^2	d.f.	*P*	Number of contaminants
	Female	Male						
872	120/120	115/120	33	18 120/120, 15 120/115	0·27	1	n.s.	0
1072	120/75	120/100	72	24 120/120, 19 120/75, 15 120/100, 14 100/75	3·45	3	n.s.	2
1080	120/120	120/100	36	20 120/100, 16 120/120	0·44	1	n.s.	0
1111	115/100	120/100	58	19 100/100, 14 120/115, 12 115/100, 13 120/100	2·00	3	n.s.	1
1114	120/100	120/115	79	14 120/120, 27 120/100, 19 120/115, 19 115/100	4·39	3	n.s.	0
1135	115/100	120/120	43	25 120/100, 18 120/115	1·14	1	n.s.	1
1211	100/100	100/75	102	55 100/75, 47 100/100	0·63	1	n.s.	0
1625	120/120	120/100	153	76 120/100, 77 120/120	0·01	1	n.s.	1
1686	120/120	120/100	37	18 120/100, 19 120/120	0·03	1	n.s.	2
1698	120/115	120/100	40	9 100/100, 16 120/115, 8 115/100, 7 120/100	5·00	3	n.s.	0
1918	100/100	120/100	162	78 120/100, 84 100/100	0·22	1	n.s.	4
1960	120/75	120/100	47	13 120/100, 14 120/120, 10 100/75, 10 120/75	1·09	3	n.s.	1
2429	120/120	120/100	54	29 120/100, 25 100/100	0·30	1	n.s.	0

Table 3 Segregation of alleles at the *Pgi* locus

Cross number	Parental genotype: Female	Parental genotype: Male	*N*	Progeny genotypes	χ^2	d.f.	*P*	Number of contaminants
872	100/98	100/98	53	16 100/100, 25 100/98, 12 98/98	0·77	2	n.s.	0
1072	100/90	90/90	70	38 100/90, 32 90/90	0·51	1	n.s.	2
1114	100/100	100/90	80	37 100/100, 43 100/90	0·45	1	n.s.	0
1686	100/100	100/90	33	18 100/100, 15 100/90	0·27	1	n.s.	2
1698	100/90	100/100	41	21 100/100, 20 100/90	0·02	1	n.s.	0
1913	100/100	100/90	62	29 100/100, 33 100/90	0·26	1	n.s.	1
1918	100/100	100/90	184	77 100/100, 107 100/90	4·89	1	0·03	4
1926	100/90	100/100	88	39 100/100, 49 100/90	1·14	1	n.s.	0
2429	100/100	100/90	54	27 100/100, 27 100/90	0·00	1	n.s.	0
2451	100/100	100/90	36	19 100/100, 17 100/90	0·11	1	n.s.	0

Analysis of 100/100 × 100/90 crosses:

	χ^2	d.f.	*P*
Overall deviation (1:1)	3·349	1	0·067
heterogeneity	3·791	7	n.s.
Total	7.140	8	n.s.

Pgi^{100}/Pgi^{90}, a surplus of heterozygotes. If progeny from all like crosses ($Pgi^{100}/Pgi^{100} \times Pgi^{100}/Pgi^{90}$) are pooled, a total of 267 Pgi^{100}/Pgi^{100} and 311 Pgi^{100}/Pgi^{90} are observed, a distribution not significantly different from the expected 1:1 ratio at the 5 per cent level ($P = 0{\cdot}067$), and there is no significant heterogeneity between crosses (table 3). Thus it seems likely that the heterozygote excess observed in the progeny of cross 1918 is a sampling artefact, and does not represent an increase in viability of the heterozygous genotype.

Data for the *Pgm*-1 and *Pgm*-2 loci are presented in table 4. A third locus, *Pgm*-F, is frequently observed in natural populations (Janson and Ward, 1984), but the products of this locus are too weak to score in laboratory reared juveniles. Eight crosses were analysed for the *Pgm*-1 locus and eleven for *Pgm*-2. All progeny ratios accorded with

Table 4 Segregation of alleles at the *Pgm*-1 and *Pgm*-2 loci

Cross number	Parental genotype: Female	Parental genotype: Male	*N*	Progeny genotypes	χ^2	d.f.	*P*	Number of contaminants
Pgm-1								
872	105/100	100/100	53	28 100/100, 25 105/100	0·17	1	n.s.	0
1072	105/85	100/100	69	32 105/100, 37 100/85	0·36	1	n.s.	2
1080	100/85	100/85	36	10 100/100, 16 100/85, 10 85/85	0·44	2	n.s.	0
1114	105/100	85/85	80	40 105/85, 40 100/85	0·00	1	n.s.	0
1135	100/85	85/85	43	23 100/85, 20 85/85	0·21	1	n.s.	1
1625	105/100	100/85	153	31 100/85, 37 100/100, 47 105/100, 38 105/85	3·42	3	n.s.	1
1698	105/100	100/100	16	7 105/100, 9 100/100	0·25	1	n.s.	0
1960	100/85	100/85	43	7 100/100, 24 100/85, 12 85/85	1.74	2	n.s.	1
Pgm-2								
872	100/100	85/85	53	53 100/85	—	—	—	0
1072	85/70	100*/70	47	12 70/70, 10 100*/70, 12 100*/85, 13 85/70	0·40	3	n.s.	2
1111	85/85	100/100	36	36 100/85	—	—	—	1
1114	100/85	100/70	80	18 85/70, 28 100/100, 18 100/85, 16 100/70	4·40	3	n.s.	0
1135	100/85	100/100	42	21 100/85, 21 100/100	0·00	1	n.s.	1
1211	100/100	85/70	99	45 100/70, 54 100/85	0·82	1	n.s.	0
1625	100/100	100/70	153	83 100/100, 70 100/70	1·10	1	n.s.	0
1698	100/85	85/85	32	14 85/85, 18 100/85	0·50	1	n.s.	0
1926	100/70	100/85	87	23 85/85, 24 100/85, 22 100/70, 18 85/70	0·95	3	n.s.	0
1960	100/85	85/85	54	26 85/85, 28 100/85	0·07	1	n.s.	1
2451	100/100	100/85	36	17 100/100, 19 100/85	0·11	1	n.s.	0

(*Pgm*-2 100*: a low activity allele)

Table 5 Segregation of alleles at the *Aat*-1, *Aat*-2, *Ap*-2, *Lap*-1, *Odh* and *Idh*-2 loci

Cross number	Parental genotype		*N*	Progeny genotypes	χ^2	d.f.	*P*	Number of contaminants
	Female	Male						
Aat-1								
1072	120/100	120/100	71	21 120/120, 36 120/100, 14 100/100	1·39	2	n.s.	2
1111	120/100	120/120	58	25 120/120, 33 120/100	1·10	1	n.s.	1
1135	100/100	120/120	43	43 120/100	—	—	—	1
1211	120/100	100/100	102	55 100/100, 47 120/100	0·63	1	n.s.	0
1625	100/100	120/100	153	82 100/100, 70 120/100	0·95	1	n.s.	0
1913	100/100	120/100	62	27 100/100, 35 120/100	1·03	1	n.s.	1
1952	120/100	100/100	87	42 100/100, 45 120/100	0·10	1	n.s.	0
2429	100/100	120/100	55	34 100/100, 21 120/100	3·07	1	n.s.	0
Aat-2								
1625	100/100	100/100	96	96 100/100	—	—	—	1
1913	?	?	48	22 100/100, 26 150/100	0·33	1	n.s.	1
2429	100/100	150/100	51	28 100/100, 23 150/100	0·49	1	n.s.	0
Ap-2								
872	100/100	100/100	14	14 100/100	—	—	—	0
1960	115/100	100/100	68	39 100/100, 29 115/100	1·47	1	n.s.	0
Lap-1								
1926	105/100	100/100	17	8 100/100, 9 105/100	0·06	1	n.s.	0
Odh								
1111	105/105	105/105	32	32 105/105	—	—	—	0
1211	105/100	105/100	102	34 105/105, 39 105/100, 29 100/100	6·14	2	0·05	0
1625	100/100	105/105	48	48 105/100	—	—	—	0
1918	105/105	105/100	130	67 105/105, 63 105/100	0·12	1	n.s.	4
1952	105/100	105/105	84	41 105/105, 43 105/100	0·05	1	n.s.	0
Idh-2								
2451	100/100	100/75	36	17 100/100, 19 100/75	0·11	1	n.s.	0

Mendelian expectations. Cross 1072 is particularly interesting with respect to the *Pgm*-2 locus. The male parent was heterozygous for the $Pgm\text{-}2^{85}$ allele and a low activity $Pgm\text{-}2^{100}$ allele, producing a markedly asymmetrical two banded phenotype. This low activity allele was inherited and expressed as such by the progeny. It appears therefore that there is genetically determined activity variation within the $Pgm\text{-}2^{100}$ allele.

Table 6 Deviations of observed heterozygosity from expected heterozygosity among the offspring of segregating crosses

Locus	*N*	$\bar{D}$	*D* (pooled)
Mpi	12	−0·0038	−0·0094
Pgi	10	0·0274	0·0670
Pgm-1	6	−0·0160	0·0036
Pgm-2	8	−0·0334	−0·0376
Aat-1	7	−0·1200	−0·0238
Aat-2	2	−0·0074	−0·0101
Ap-2	1	−0·1471	−0·1471
Lap-1	1	0·0588	0·0588
Odh	3	−0·0346	−0·0523
Idh-2	1	0·0556	0·0556

N = number of segregating crosses. $\bar{D}$ = mean of the *N* values of *D* where $D = (Hob - Hexp)/Hexp$ and where *H*ob and *H*exp are the numbers of heterozygotes observed and expected respectively in each cross. *D*(pooled) = value of *D* calculated from the pooled numbers of heterozygotes over every cross at that locus.

Eight crosses were analysed for *Aat*-1 (table 5), and in each case, progeny numbers accorded with Mendelian expectations. With respect to *Aat*-2, progeny were observed to be segregating in a cross (1913) whose parents had not, unfortunately, been scored for this locus. However, these progeny segregated in a 1:1 ratio, and it seems likely that they resulted from an $Aat\text{-}2^{100}/Aat\text{-}2^{100} \times Aat\text{-}2^{100}/Aat\text{-}2^{115}$ cross. Progeny analysis of a later cross whose parents had been typed (cross 2429) showed normal segregation.

Single crosses segregating for alleles at the *Ap*-2, *Lap*-1 and *Idh*-2 loci were scored, and progeny numbers accorded with expectations (table 5).

Three crosses were segregating for alleles at the *Odh* locus (table 5) and progeny crosses in two of these accorded with expectation. However, in the remaining cross (1211), a just significant deviation was observed ($P = 0{\cdot}046$). Here, the two parents were both of the Odh^{100}/Odh^{105} genotype, and the progeny numbers (with expected values) were as follows: 29 (25·5) Odh^{100}/Odh^{100}, 39 (51) Odh^{100}/Odh^{105}, 34 (25·5) Odh^{105}/Odh^{105}. This deviation may have arisen because the male parent had an abnormally low activity Odh^{100} allele, so

that some Odh^{100}/Odh^{105} heterozygotes may have been mistakenly scored as Odh^{105}/Odh^{105} homozygotes, or it might simply have arisen through sampling error.

There is no indication of a general excess of homozygous or heterozygous individuals amongst the progeny of segregating crosses. This data is summarised in table 6. The total observed and expected numbers of heterozygotes and homozygotes respectively are 1937 and 1947·25, and 1626 and 1615·75. These differences are non-significant ($\chi^2_1 = 0{\cdot}119$, $P > 0{\cdot}05$).

Linkage relationships

Linkage relationships between pairs of loci could be examined in two types of crosses: (a) where one parent was heterozygous for the two loci in question and the other parent homozygous, and (b) where one parent was heterozygous for the two loci and the other parent differently heterozygous at one locus and homozygous at the other.

Type 1:

e.g., Cross 1072 ♀ *Pgi* 100/90 *Pgm*-1 105/85 × ♂ *Pgi* 90/90 *Pgm*-1 100/100

↓

Set A *Pgi* 100/90 *Pgm*-1 105/100, *Pgi* 90/90 *Pgm*-1 85/100.

Set B *Pgi* 100/90 *Pgm*-1 85/100, *Pgi* 90/90 *Pgm*-1 105/100.

If *Pgi* and *Pgm*-1 were linked, and if in the female parent the Pgi^{100} and $Pgm\text{-}1^{105}$ alleles were in coupling, then the first two genotype classes (Set A) would be the parental types, and the second two genotypes (Set B) would be recombinants. Since the animals used in these crosses have come from wild populations and are of unknown linkage phase, we test for linkage by comparing the total number in set A with the total number in set B, producing a chi-square with a single degree of freedom. In this cross, the total chi-square for a 1 : 1 : 1 : 1 segregation has three degrees of freedom.

Type 2:

e.g., Cross 1072 *Pgi* ♀ 100/90 *Mpi* 120/75 × ♂ *Pgi* 90/90 *Mpi* 120/100

↓

Set A *Pgi* 100/90 *Mpi* 120/120,
Pgi 100/90 *Mpi* 120/100,
Pgi 90/90 *Mpi* 120/75,
Pgi 90/90 *Mpi* 100/75.

Set B *Pgi* 100/90 *Mpi* 120/75,
Pgi 100/90 *Mpi* 100/75,
Pgi 90/90 *Mpi* 120/120,
Pgi 90/90 *Mpi* 120/100.

Again, the test for linkage is to compare the total number in set A with the total number in set B, to give a chi-square with one degree of freedom, and the total chi-square for a 1 : 1 : 1 : 1 : 1 : 1 : 1 : 1 segregation has seven degrees of freedom.

For convenience, we have designated the most populous set as set A in all our crosses.

Five loci (*Pgi, Mpi, Pgm*-1, *Pgm*-2, *Aat*-1) were studied for all possible pairwise linkage comparisons, and the remaining five loci (*Ap*-2, *Odh*, *Lap*-1, *Aat*-2 and *Idh*-2) enabled some additional comparisons to be made. A summary of results is provided in table 7, with a fuller presentation in the Appendix.

Of the five loci that could be studied for all possible comparisons, only the *Mpi* and *Aat*-1 loci gave results indicative of loose linkage. The three crosses gave linkage chi-square values of 6·90 ($P = 0{\cdot}009$), 1·68 ($P = 0{\cdot}195$), and 1·85 ($P = 0{\cdot}174$) respectively, summing to 10·43 (3 d.f., $P = 0{\cdot}015$). The recombination fraction, assuming the set A individuals are the parental types, is 0·41 (109/264). Unfortunately, since we cannot be certain that the set A individuals are parental types, this recombination fraction must be regarded as provisional, and we cannot partition the summed chi-square into deviation and heterogeneity components.

With respect to the *Mpi* : *Pgi* and *Mpi* : *Pgm*-1 locus pairs, one out of six and one out of five progeny sets respectively gave significant linkage chi-square values, but for both pairs of loci the summed chi-square values are non-significant ($\chi^2 = 11{\cdot}58$, 6 d.f., $P = 0{\cdot}07$, and $\chi^2 = 7{\cdot}09$, 5 d.f., $P = 0{\cdot}214$). Three further pairs of loci showed some signs of linkage (*Pgi* : *Lap*-1, *Pgi* : *Odh*, *Aat*-1 : *Odh*) but in each case the χ^2 value is only just significant at $P = 0{\cdot}05$, and in each case only a single cross yields usable data.

Two crosses used *L. arcana* females. The 1211 cross looked at *Mpi* : *Pgm*-2 linkage, and the *arcana* here was doubly homozygous. The 1625 cross looked at linkage between *Pgm*-1, *Pgm*-2 and *Aat*-1, and only for the *Pgm*-1 locus was the *arcana* heterozygous (as was the male *saxatilis*). Thus these crosses can justifiably be claimed to be looking at linkage in the *saxatilis* genome.

Table 7 Summary of linkage studies. Cross numbers enabling particular pairwise linkage comparisons to be studied are given, and those where the linkage chi-square value is significant are indicated. See appendix for further details

	Pgi	*Pgm*-1	*Pgm*-2	*Aat*-1	*Aat*-2	*Ap*-2	*Lap*-1	*Odh*	*Idh*-2
Mpi	1072 1114 1686 1698 1918† 2429	1072 1114* 1135 1625 1698	1135 1211 1625 1698 1960	1111† 1625 2429	2429	1960	*n.d.*	1918	*n.d.*
Pgi		1072 1698	1072 1114 1698 1926 2451	1913 2429	2429	*n.d.*	1926*	1918*	2451
Pgm-1			1072 1114 1135 1625 1698	1625	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Pgm-2				1625	*n.d.*	1960	1926	*n.d.*	2451
Aat-1					2429	*n.d.*	*n.d.*	1952*	*n.d.*
Aat-2						*n.d.*	*n.d.*	*n.d.*	*n.d.*
Ap-2							*n.d.*	*n.d.*	*n.d.*
Lap-1								*n.d.*	*n.d.*
Odh									*n.d.*

* $P = 0{\cdot}05 - 0{\cdot}01$.
† $P = 0{\cdot}01 - 0{\cdot}001$.
n.d. = no data.

DISCUSSION

Segregation of alleles at all 10 of the polymorphic loci examined (*Mpi*, *Pgi*, *Pgm*-1, *Pgm*-2, *Aat*-1, *Aat*-2, *Ap*-2, *Lap*-1, *Odh*, *Idh*-2) in *Littorina saxatilis* followed Mendelian expectations, and thus earlier genetic interpretations of gel patterns can be regarded as valid. Variants at an eleventh locus, purine nucleoside phosphorylase (*Np*) have also been shown to segregate normally (Knight and Ward, 1986). If the ten loci described in the present paper are considered, 45 pairwise linkage comparisons are possible; at present we have data on 22 of these. Four pairs show some evidence of loose linkage, but further data are required to confirm these findings; none of the pairs showed any evidence of tight linkage. The two loci showing the strongest evidence of linkage are *Aat*-1 and *Mpi*, but even here the results are not conclusive. *Littorina saxatilis* has a haploid count of 17 chromosomes (Janson, 1983), so the lack of evidence for strong linkage is not perhaps surprising. On the other hand, the *a priori* probability that all 10 of these loci are on separate chromosomes is low ($P = 0{\cdot}035$).

Mallett *et al.*, (1985) observed that at four of six loci screened in the progeny of the mussel, *Mytilus edulis*, the overall number of heterozygotes was significantly less than that expected from the parental genotypes. They proposed that genotype-dependent larval mortality was the primary cause of the heterozygote deficits frequently observed in natural populations of this mollusc. Deviations from Hardy–Weinberg expectations are uncommon in *L. saxatilis* (Ward and Warwick, 1980; Janson and Ward, 1984), and, in the laboratory crosses detailed here, there are no indications of significant genotype-dependent larval mortality.

This paper also shows that female *L. arcana* will cross with male *L. saxatilis*, at least in the laboratory, and viable offspring are produced. However, attempts at carrying out the reciprocal cross have been unsuccessful. Although these two species have differing reproductive modes, *saxatilis* being ovoviparous and *arcana* oviparous, they are morphologically (Hannaford Ellis, 1979) and genetically (Ward and Warwick, 1980; Ward and Janson, 1985) closely related, with a genetic identity value of around 0·95. No diagnostic locus has yet been identified to facilitate study of the reproductive dynamics of these two species when sympatric, but the differences in gene frequency found in sympatric populations (Ward and Warwick, 1980; Ward and Janson, 1985) seem unlikely to result from physical or gametic barriers to the production of hybrids. We do not yet know whether hybrid individuals are sterile or fertile.

Breeding experiments are continuing to investigate further enzyme loci, including the complex esterases, and animals of known linkage phase are being produced to act as parents in future generations. The genetics of shell morphology (colours, banding patterns, reticulations) are also being studied.

Acknowledgements Our thanks are due to the following people who kindly collected material, N. E. Buxton, D. S. Davis, J. Fair, K. Gilkinson, M. McAuley, R. O. M. Page. Part of this work was supported by research grant GR3/5319 from the Natural Environment Research Council to R. D. Ward.

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APPENDIX

Details of the breeding experiments enabling linkage comparisons to be made

Locus pair	Cross	Number of progeny			Linkage chi (1 d.f.)	r (%)	Total	
		Total	A	B			chi	d.f.
Pgi: *Mpi*	1072	70	38	32	0·514	—	9·543	7
	1114	79	40	39	0·013	—	5·962	7
	1686	32	19	13	1·125	—	1·250	3
	1698	40	22	18	0·400	—	8·000	7
	1918	162	99	63	8·000†	38·9	11·778*	3
	2429	53	31	22	1·528	—	1·717	3
Pgi: *Pgm*-1	1072	67	37	30	0·731	—	2·313	3
	1698	16	8	8	0	—	0·500	3
Pgi: *Pgm*-2	1072	45	23	22	0·022	—	2·467	7
	1114	80	41	39	0·050	—	6·200	7
	1698	32	17	15	0·125	—	0·750	3
	1926	87	47	40	0·563	—	3·023	7
	2451	36	20	16	0·444	—	0·667	3
Pgi: *Aat*-1	1913	62	34	28	0·580	—	1·871	3
	2429	54	31	23	1·185	—	4·815	3
Pgi: *Aat*-2	2429	50	31	19	2·880	—	3·280	3
Pgi: *Lap*-1	1926	17	13	4	4·765*	23·5	4·882	3
Pgi: *Odh*	1918	130	77	51	4·431*	40·8	7·046	3
Pgi: *Idh*-2	2451	36	20	16	0·444	—	0·667	3
Mpi: *Pgm*-1	1072	69	38	31	0·710	—	2·467	7
	1114	79	50	29	5·582*	36·7	10·215	7
	1135	43	23	20	0·209	—	1·558	3
	1625	153	81	72	0·529	—	1·529	3
	1698	15	8	7	0·067	—	10·067	7
Mpi: *Pgm*-2	1135	42	25	17	1·524	—	2·381	3
	1211	99	54	45	0·818	—	2·859	3
	1625	153	78	75	0·059	—	1·170	3
	1698	31	16	15	0·032	—	9·516	7
	1960	38	21	17	0·381	—	7·053	7
Mpi: *Aat*-1	1111	58	39	19	6·897†	32·7	10·690	7
	1625	152	84	68	1·68	—	2·632	3
	2429	54	32	22	1·85	—	4·815	3
Mpi: *Aat*-2	2429	51	31	20	2·37	—	3·353	3
Mpi: *Ap*-2	1960	41	21	20	0·024	—	2·512	7
Mpi: *Odh*	1918	130	67	63	0·123	—	0·277	3
Pgm-2: *Pgm*-2	1072	44	24	20	0·364	—	4·727	3
	1114	80	44	36	0·800	—	9·600	7
	1135	42	25	17	1·524	—	1·619	3
	1625	153	77	76	0·007	—	4·856	7
	1698	16	9	7	0·250	—	1·500	3
Pgm-1: *Aat*-1	1625	152	84	68	1·684	—	7·789	7
Pgm-2: *Aat*-1	1625	152	82	70	0·947	—	2·842	3
Pgm-2: *Ap*-2	1960	50	25	25	0	—	0·400	3
Pgm-2: *Lap*-1	1926	17	9	8	0·059	—	1·588	7
Pgm-2: *Idh*-2	2451	36	22	14	1·778	—	2·000	3
Aat-1: *Odh*	1952	84	51	33	3·857*	39·3	4·095	3
Aat-1: *Aat*-2	2429	51	29	22	0·961	—	3·039	3

r = recombination fraction.
* $P = 0{\cdot}05 - 0{\cdot}01$.
† $P = 0{\cdot}01 - 0{\cdot}001$.