Heredity **66** (1991) 151–158

Received 13 June 1990

Further genetic analysis of polymorphic enzyme loci in *Littorina saxatilis* (Prosobranchia: Mollusca)

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Electrophoretic variants of seven loci (*Pgi*, *Mpi*, *Pgm-1*, *Pgm-2*, *Aat-1*, *Np* and *Odh*) have been screened, in all possible pairwise combinations, in laboratory crosses of *Littorina saxatilis*. Two loci, *Pgi* and *Np*, are shown to be linked with a maximum likelihood recombination frequency of 0.30. Recombination occurs in both males and females. No strong evidence for linkage between other pairs of loci, including miscellaneous comparisons involving seven additional loci (*Aat-2*, *Idh-1*, *Idh-2*, *Lap-1*, *Ap-2*, *Apk* and *Hdh*), was obtained. Variants at all 14 polymorphic loci segregate in a Mendelian manner.

Keywords: electrophoretic variants, *Littorina saxatilis*, polymorphic loci.

Introduction

Snails of the intertidal genus *Littorina* have been the subject of many population genetic studies in the last decade (reviewed in Ward, 1990). Most of these studies have utilized the electrophoretic analysis of soluble enzymes, and it is reassuring that, for many of these enzymes in *Littorina saxatilis* (= *L. rudis*), the phenotypic variation observed in allozyme banding patterns has been demonstrated (by laboratory breeding experiments) to have a simple genetic basis (Knight & Ward, 1986; Ward *et al.*, 1986). These earlier genetic studies have now been significantly extended, both by further work on *L. saxatilis* and, to a lesser extent, by studies on its closely related (Ward & Warwick, 1980; Knight & Ward, 1990) sibling species *L. arcana*. Mendelian inheritance of three additional polymorphisms has been confirmed (arginine phosphokinase, isocitrate dehydrogenase-1, and hexanol dehydrogenase), making 14 polymorphisms in total with a demonstrated genetic basis. In addition, seven loci have been examined in all possible pairwise comparisons for evidence of chromosomal linkage, and many other pairwise combinations of a further seven loci have also been examined. Two loci, *Np* (nucleoside phosphorylase) and *Pgi* (phosphoglucose isomerase), are linked.

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Materials and methods

Methods for breeding snails of the *L. saxatilis* complex (*L. saxatilis*, *L. arcana*, *L. neglecta* and *L. nigrolineata*) are given by Warwick (1983). Attempts to hybridize members of the *L. saxatilis* complex in the laboratory are described by Warwick *et al.* (1990).

Electrophoretic methods and locus and allele designations follow Ward & Warwick (1980) and Janson & Ward (1984). The 14 loci scored in the breeding experiments are: phosphoglucose isomerase (*Pgi*, E.C. 5.3.1.9), mannose phosphate isomerase (*Mpi*, E.C. 5.3.1.8), isocitrate dehydrogenases -1 and -2 (*Idh-1*, *Idh-2*, E.C. 1.1.1.42), aspartate aminotransferases -1 and -2 (*Aat-1*, *Aat-2*, E.C. 2.6.1.1), phosphoglucomutases -1 and -2 (*Pgm-1*, *Pgm-2*, E.C. 2.7.5.1), octanol dehydrogenase (*Odh*, E.C. 1.1.1.73), hexanol dehydrogenase (*Hdh*, E.C. 1.1.1.?), nucleoside phosphorylase (*Np*, E.C. 2.4.2.1), arginine phosphokinase (*Apk*, E.C. 2.7.3.3), leucine aminopeptidase-1 (*Lap-1*, E.C. 3.4.11.1/2) and aminopeptidase-2 (*Ap-2*, E.C. 3.4.11.?).

Results

The origins of the males and virgin females used in the crosses are given in Table 1. While the majority of the crosses were between *L. saxatilis* males and females, two crosses, which generated electrophoretically screened progeny, used female *arcana* and male

Table 1 Sources and species of animals used in breeding experiments

Cross	Source	Species
872	♀ Loch Bee, South Uist, Scotland	*saxatilis*
	♂ 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*
1879	♀ Oban a Chlachain, North Uist, Scotland	*saxatilis*
	♂ Village Bay, Hirta, St Kilda, Scotland	*saxatilis*
1893	♀ Basalt, Milsey Bay, North Berwick, Scotland	*arcana*
	♂ Cargreen, Scotland	*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*
1960	♀ Newford Island, St Mary's, Scilly Isles, England	*saxatilis*
	♂ Penzance (beach), Cornwall, England	*saxatilis*
2429	♀, ♂ Marine Villa, Dirleton (beach), Scotland	*saxatilis*
2433	♀ Marine Villa, Dirleton (beach), Scotland	*saxatilis*
	♂ Fleet at Moonfleet, Dorset, England	*saxatilis*
2448	♀ an F_1 progeny from cross 1879	*saxatilis*
	♂ Village Bay, Hirta, St Kilda, Scotland	*saxatilis*
2451	♀ an F_1 progeny from cross 1879	*saxatilis*
	♂ Village Bay, Hirta, St Kilda, Scotland	*saxatilis*
2453	♀ an F_1 progeny from cross 1879	*saxatilis*
	♂ Village Bay, Hirta, St Kilda, Scotland	*saxatilis*
2520	♀ Inverkirkaig, Sutherland, Scotland	*saxatilis*
	♂ Logan Road, Isle of May, Scotland	*saxatilis*
2522	♀ Inverkirkaig, Sutherland, Scotland	*saxatilis*
	♂ Logan Road, Isle of May, Scotland	*saxatilis*
2553	♀ Seacliff, North Berwick, Scotland	*saxatilis*
	♂ Logan Road, Isle of May, Scotland	*saxatilis*
2555	♀ Inverkirkaig, Sutherland, Scotland	*saxatilis*
	♂ Logan Road, Isle of May, Scotland	*saxatilis*
2927	♀ an F_1 progeny from cross 1893	*hybrid*
	♂ Aberlady (salting), East Lothian, Scotland	*saxatilis*
3278	♀ Johnson's Hole, Dunbar Harbour, Scotland	*arcana*
	♂ Roxburgh Hotel (wall), Dunbar, Scotland	*arcana*
3283	♀ Johnson's Hole, Dunbar Harbour, Scotland	*arcana*
	♂ Roxburgh Hotel (wall), Dunbar, Scotland	*arcana*

Table 1 *Continued.*

Cross	Source	Species
3284	♀ Johnson's Hole, Dunbar Harbour, Scotland	*arcana*
	♂ Roxburgh Hotel (wall), Dunbar, Scotland	*arcana*
3287	♀, ♂ Roxburgh Hotel (wall), Dunbar, Scotland	*arcana*
3290	♀, ♂ Roxburgh Hotel (wall), Dunbar, Scotland	*arcana*
3655	♀ Hudson Bay, Canada	*saxatilis*
	♂ Tongue, Sutherland, Scotland	*saxatilis*
3656	♀ Hudson Bay, Canada	*saxatilis*
	♂ Tongue, Sutherland, Scotland	*saxatilis*

Table 2 Segregation of alleles at the *Idh-1*, *Apk*, *Hdh* and *Np* loci

	Parental genotype						
Cross	Female	Male	*n*	Progeny genotypes	χ^2	d.f.	*P*
Idh-1							
3655	100/100	125/100	82	37 125/100, 45 100/100	0.78	1	ns
3656	100/100	125/100	124	56 125/100, 68 100/100	1.16	1	ns
*ApK**							
3278†	m/m	m/s	13	5 m/m, 8 m/s	0.69	1	ns
3283†	m/s	m/m	35	16 m/m, 19 m/s	0.26	1	ns
Hdh							
3278†	145/100	145/145	13	8 145/145, 5 145/100	0.69	1	ns
3290†	145/145	145/100	29	17 145/145, 12 145/100	0.86	1	ns
Np							
2448	100/35	100/65	45	10 100/100, 13 100/65, 10 100/35, 12 65/35	0.60	3	ns
2453	100/35	100/100	42	15 100/100, 27 100/35	3.42	1	ns
2520	100/65	65/65	29	15 100/65, 14 65/65	0.03	1	ns
2522	65/65	100/65	28	14 100/65, 14 65/65	0	1	ns
2553	65/65	100/65	30	10 100/65, 20 65/65	3.33	1	ns
3655	100/35	100/65	79	22 100/100, 11 100/65, 26 100/35, 20 65/35	6.11	3	ns
3656‡	100/65	65/65	125	73 100/65, 52 65/65	3.53	1	ns

*The reference population for designating allele homologies (*L. arcana* from Roxburgh Hotel sea wall, Dunbar, Scotland) has not yet been screened for *Apk*, hence the alphabetic rather than numeric allele designations.
†*L. arcana* × *L. arcana* crosses.
‡A single animal with an *Np* phenotype (100/35) inconsistent with its presumptive parents was detected: this is assumed to be a contaminant.

saxatilis (crosses 1211 and 1625: in both cases the double heterozygote was the *saxatilis* male). Five crosses used *arcana* animals as both parents. The latter crosses were generally used to analyse loci that show low-level variation in *saxatilis* populations.

Segregation of alleles at single loci

Segregation of alleles at 11 of the loci (*Pgi*, *Mpi*, *Idh-2*, *Aat-1*, *Aat-2*, *Pgm-1*, *Pgm-2*, *Odh*, *Lap-1*, *Ap-2* and *Np*) had earlier been shown to follow Mendelian expectations (Knight & Ward, 1986; Ward *et al.*, 1986). Table 2 provides segregation data for three loci studied for the first time (*Idh-1*, *Hdh* and *Apk*) and additional information for the previously little studied locus *Np*. No significant deviations from Mendelian expectations for any of these loci were seen, and although several of the seven *Np* crosses came close to significance at the 5 per cent level, the combined *Np* chi-square (17.02, with 11 d.f.) was not significant ($P = 0.11$).

Table 3 Summary of linkage studies. Cross numbers enabling particular pairwise linkage comparisons to be studied are given, and those where the linkage chi-square value (1 d.f.) is significant are indicated. *L. arcana* × *L. arcana* cross numbers are given in italics

	Pgi	*Pgm-1*	*Pgm-2*	*Aat-1*	*Np*	*Odh*
All pairwise combinations of *Pgi*, *Mpi*, *Pgm-1*, *Pgm-2*, *Aat-1*, *Np* and *Odh*						
Mpi	1072 1114 1686 1698 1918** 2429 2553 2555 2927*	1072 1114* 1135 1625 1698	1135 1211 1625 1698 1960 2520	1111** 1625 2429 2555* *3284* 3655	2553 3655	1918
Pgi		1072 1698 2433	1072 1114 1698 1926 2433 2448 2451 2520 3655	1913 2429 2555	2448** 2520 2553 3655***	1918* 2433
Pgm-1			1072 1114 1135 1625 1698 2433 2553	1625 2433* *3287*	2448 2453	2433 2453
Pgm-2				1625 2433 3656	2448 2520 3655 3656	2433 2520
Aat-1					2522 3655	1952*
Np						2453

Miscellaneous pairwise comparisons

Pgi	*Aat-2*	2429	*Pgm-2*	*Hdh*	3290
Pgi	*Idh-1*	3655	*Pgm-2*	*Idh-1*	3655, 3656
Pgi	*Idh-2*	2451, 2520, 2553	*Pgm-2*	*Idh-2*	2451, 2520
Pgi	*Lap-1*	1926*	*Pgm-2*	*Lap-1*	1926
Pgi	*Apk*	3278	*Pgm-2*	*Ap-2*	1960
Mpi	*Aat-2*	2429	*Aat-1*	*Aat-2*	2429
Mpi	*Idh-2*	2553, 2555	*Aat-1*	*Idh-1*	3656
Mpi	*Ap-2*	1960			
Pgm-1	*Hdh*	3278	*Np*	*Idh-1*	3655
Pgm-1	*Apk*	3278, 3283	*Np*	*Idh-2*	2520*, 2553

Probability levels: *$P = 0.05$–0.01, **$P = 0.01$–0.001, ***$P < 0.001$.

Linkage analysis

As outlined in Ward *et al.* (1986), two types of cross are suitable to examine linkage relationships between pairs of loci: one in which one parent is heterozygous for both loci and the other homozygous; and a second where one parent is heterozygous for both loci and the other differently heterozygous at one locus and homozygous at the other. In both cases, it is possible to ascribe offspring to one of two types of class, one of which will be the recombinant and the other the non-recombinant.

Comparing numbers in each class gives a linkage chi-square with one degree of freedom. Seven loci (*Mpi*, *Pgi*, *Pgm-1*, *Pgm-2*, *Aat-1*, *Np* and *Odh*) have now been analysed in all possible pairwise combinations and data are available for seven further loci (*Aat-2*, *Idh-1*, *Idh-2*, *Lap-1*, *Ap-2*, *Hdh* and *Apk*) studied in miscellaneous combinations. A summary of crosses is provided in Table 3.

Fifteen pairwise locus comparisons were studied only in single crosses. Two of these gave significant chi-squares indicative of loose linkage (*Pgi/Lap-1*, $\chi^2 = 4.77$, $P = 0.030$ and *Aat-1/Odh*, $\chi^2 = 3.86$, $P = 0.049$) but these indications need to be confirmed using additional crosses.

Twenty-four comparisons were studied in multiple crosses. For each of these comparisons, linkage chi-square values were summed and probability levels estimated. Three of these comparisons proved interesting. Most striking was the *Pgi/Np* locus pair. The four crosses contributing data gave linkage chi-square values of 8.02 ($P = 0.004$), 1.69 ($P = 0.194$), 2.13 ($P = 0.094$) and 19.25 ($P < 0.001$), yielding a combined chi-square of 31.09 (d.f. = 4, $P < 0.001$). Evidence of linkage was also obtained for the *Aat-1/Mpi* comparison (summed $\chi^2 = 15.73$, d.f. = 6, $P = 0.015$; ignoring the *arcana* × *arcana* cross, summed $\chi^2 = 15.13$, d.f. = 5, $P = 0.010$) and, less convincingly, for the *Pgi/Mpi* pair (summed $\chi^2 = 17.87$, d.f. = 9, $P = 0.037$). Unfortunately, because parental snails were in most cases taken from the wild, linkage phase relationships are unknown and it is not possible to be certain which progeny are recombinants and which are non-recombinants. Assuming that the loci are linked and that therefore the smaller progeny set in each case represents the recombinants, the recombination fractions for these three pairwise comparisons are: *Pgi/Np* 0.30, *Aat-1/Mpi* 0.42 (0.41 ignoring the *arcana* × *arcana* cross), and *Pgi/Mpi* 0.43. These values must be regarded as provisional.

It would clearly be advantageous to be able to combine sets of progeny from different crosses in a more satisfactory manner. One such method is to use maximum likelihood approaches to estimate the probability of recombination (θ) for a range of recombination frequencies. The maximum likelihood estimate of θ is the value at which the probability peaks. Values of θ are then compared with the probability that the two loci are unlinked in the particular pedigree, and the logarithm (to base 10) of the ratio of the two probabilities is called the z or LOD (logarithm of the odds) score at that value of θ. Given several pedigrees, the z scores can be accumulated over pedigrees (for each of a range of θ values) and the sum (Z) used to obtain the likelihood estimate. Thus a Z score of 1 corresponds to odds of 10:1 in favour of the hypothesis of linkage versus independence. However, because non-linkages are expected to be many times more common than linkages, it has been stressed that in humans (for example), a Z or LOD score of 3 corresponds not to 1000:1 odds in favour of linkage, but to 20:1 as non-linkages are about 50 times more common than linkages. Thus in humans a Z score of at least 3 is required for simple detection of linkage (Morton, 1955; Lander, 1988). Heterogeneity of linkage estimates among crosses can be tested using Morton's (1956) approach, which yields a statistic distributed approximately as a chi-square with the number of degrees of freedom equal to the number of independent pedigrees minus one.

This approach, as outlined by Cavalli-Sforza & Bodmer (1971), has been used here further to examine linkage relationships in *L. saxatilis* (Tables 4 and 5). Values of θ ranging from 0.1 to 0.5 in steps of 0.01 were examined. Of the seven loci examined in all pairwise comparisons, the only pair showing definite evidence of linkage is *Pgi* and *Np*. Here the Z score, for the maximum likelihood estimate of θ of 0.3, was 5.3, signifying very high odds for linkage. There was no significant heterogeneity of z scores among the four crosses that contributed data, and thus these two loci are certainly linked, albeit rather loosely. In three of the four crosses, the male parent was doubly heterozygous (2448, 2553 and 3655) but in the fourth the female was heterozygous (2520). All these crosses produced recombinant progeny, thus recombination occurs in both sexes.

There is also weak evidence for *Pgi* being linked to *Mpi* ($\theta = 0.44$, $Z = 0.87$, odds of 7.4:1), *Aat-1* being linked to *Mpi* ($\theta = 0.43$, $Z = 0.78$, odds of 6.0:1; ignoring the *arcana* × *arcana* cross, $\theta = 0.43$, $Z = 0.96$, odds of 9.1:1) and *Pgi* being linkd to *Odh* ($\theta = 0.41$, $Z = 0.67$, odds of 4.7:1). While these results are consistent with the chi-square analysis, usually much higher odds are required to be reasonably certain of linkage. Furthermore, other pairwise locus studies within the *Mpi*, *Pgi*, *Odh*, *Aat-1* and *Np* grouping do

Table 4 Maximum likelihood analysis of linkage between all possible pairwise comparisons of *Pgi*, *Mpi*, *Pgm-1*, *Pgm-2*, *Aat-1*, *Np* and *Odh*

Loci		Number		Maximum likelihood estimate of θ	*Z* score	Odds	Heterogeneity	
		Crosses	Progeny				χ^{2*}	*P*
Pgi	*Mpi*	9	520	0.44 ± 0.02	0.87	7:1	8.38	ns
Pgi	*Pgm-1*	4	120	0.50 ± 0.09	0.00	1:1	0.62	ns
Pgi	*Pgm-2*	9	458	0.50 ± 0.03	0.00	1:1	0.09	ns
Pgi	*Aat-1*	3	197	0.50 ± 0.07	0.00	1:1	0.04	ns
Pgi	*Np*	4	183	0.30 ± 0.03	5.30	2×10^5:1	2.41	ns
Pgi	*Odh*	2	158	0.41 ± 0.04	0.67	5:1	0.87	ns
Mpi	*Pgm-1*	5	359	0.46 ± 0.04	0.13	1:1	3.67	ns
Mpi	*Pgm-2*	6	379	0.50 ± 0.04	0.00	1:1	1.19	ns
Mpi	*Aat-1*	6	413	0.43 ± 0.03	0.78	6:1	6.18	ns
		5†	372	0.42 ± 0.03	0.96	9:1	5.35	ns
Mpi	*Np*	2	106	0.46 ± 0.08	0.02	1:1	0.22	ns
Mpi	*Odh*	1	130	0.50 ± 0.05	0.00	1:1	—	—
Pgm-1	*Pgm-2*	7	413	0.50 ± 0.04	0.00	1:1	1.29	ns
Pgm-1	*Aat-2*	3	225	0.45 ± 0.04	0.13	1:1	2.68	ns
		2†	176	0.43 ± 0.04	0.26	2:1	2.08	ns
Pgm-1	*Np*	2	85	0.50 ± 0.06	0.00	1:1	0.00	ns
Pgm-1	*Odh*	2	32	0.50 ± 0.20	0.00	1:1	0.11	ns
Pgm-2	*Aat-1*	3	290	0.50 ± 0.05	0.00	1:1	0.00	ns
Pgm-2	*Np*	4	239	0.49 ± 0.18	0.00	1:1	1.29	ns
Pgm-2	*Odh*	2	44	0.41 ± 0.09	0.07	1:1	0.64	ns
Aat-1	*Np*	2	106	0.50 ± 0.20	0.00	1:1	0.09	ns
Aat-1	*Odh*	1	84	0.39 ± 0.05	0.54	3:1	—	—
Np	*Odh*	1	14	0.41 ± 0.25	0.01	1:1	—	—

*Number of degrees of freedom associated with the heterogeneity chi square is number of crosses minus one.
†Excluding the *L. arcana* × *L. arcana* cross.

not yield high *Z* scores, and it seems improbable that all five loci form part of a single linkage group.

The 19 miscellaneous pairwise comparisons listed in Table 5 show a single suggestion of weak linkage, that which exists between *Pgi* and *Lap-1*. Here the odds are 6.2:1 in favour of linkage but only a single set of progeny has been analysed, and unfortunately the *Np* and *Lap-1* comparison has not been studied.

Discussion

A total of 14 polymorphic loci was examined for allelic segregation, and in each case it is clear that the banding patterns seen on gels in population surveys of *L. saxatilis* do reflect genetic variation rather than environmentally induced artifacts.

Among 14 loci, there are 91 possible pairwise combinations that can be examined for linkage, but so far only 47 have been analysed. However, seven loci have been examined in all possible pairwise combinations and of these only one pair, *Np* and *Pgi*, show unambiguous evidence of linkage. These two loci are loosely linked, with a recombination frequency of 0.30, and recombination occurs in both males and females. The haploid chromosome count of *L. saxatilis* is 17 (Janson, 1983), and thus when dealing with these seven loci, it is not surprising that definite evidence of linkage was forthcoming for only a single pair.

Another linkage group may comprise *Aat-1* and *Mpi*. The existence of linkage here receives quite strong support from the chi-square analysis, but weaker support from the maximum likelihood

Table 5 Maximum likelihood analysis of linkage between miscellaneous pairwise comparisons

Loci		Number		Maximum likelihood estimate of θ	Z score	Odds	Heterogeneity	
		Crosses	Progeny				χ^{2*}	P
Pgi	*Aat-2*	1	50	0.38 ± 0.07	0.33	2:1	—	—
Pgi	*Idh-1*	1	82	0.46 ± 0.10	0.01	1:1	—	—
Pgi	*Idh-2*	3	147	0.50 ± 0.05	0.00	1:1	0.00	ns
Pgi	*Lap-1*	1	17	0.24 ± 0.10	0.79	6:1	—	—
Pgi	*Apk*	1	13	0.50 ± 0.14	0.00	1:1	—	—
Mpi	*Aat-2*	1	51	0.39 ± 0.07	0.22	2:1	—	—
Mpi	*Idh-2*	2	55	0.37 ± 0.07	0.31	2:1	0.00	ns
Mpi	*Ap-2*	1	41	0.50 ± 0.08	0.00	1:1	—	—
Pgm-1	*Hdh*	1	13	0.50 ± 0.25	0.00	1:1	—	—
Pgm-1	*Apk*	2	47	0.50 ± 0.12	0.00	1:1	0.62	ns
Pgm-2	*Hdh*	1	29	0.50 ± 0.09	0.00	1:1	—	—
Pgm-2	*Idh-1*	2	182	0.50 ± 0.00	0.00	1:1	0.00	ns
Pgm-2	*Idh-2*	2	147	0.50 ± 0.30	0.00	1:1	0.46	ns
Pgm-2	*Lap-1*	1	17	0.50 ± 0.13	0.00	1:1	—	—
Pgm-2	*Ap-2*	1	50	0.50 ± 0.07	0.00	1:1	—	—
Aat-1	*Aat-2*	1	51	0.50 ± 0.34	0.00	1:1	—	—
Aat-1	*Idh-1*	1	123	0.46 ± 0.07	0.03	1:1	—	—
Np	*Idh-1*	1	78	0.43 ± 0.06	0.11	1:1	—	—
Np	*Idh-2*	2	59	0.37 ± 0.06	0.54	3:1	4.29	0.04

*Number of degrees of freedom equals number of crosses minus one.

approach. If these two loci are linked, linkage is very loose with a recombination frequency of around 0.42. Examining both the chi-square and likelihood approaches, there was suggestive, though far from compelling, evidence for loose linkage between a few more pairs of loci (*Pgi* and *Mpi*, *Pgi* and *Odh*, *Pgi* and *Lap-1*). None of the pairwise comparisons analysed showed any indications of tight linkage.

A single case of heterogeneity in the z score estimates was recorded. The locus pair involved was *Np* and *Idh-2*. Two sets of progeny were recorded, one showing seven progeny in one offspring class and 22 in the other (giving a maximum θ of 0.24, $z = 1.47$, corresponding to an odds for linkage of 30:1), the other 15 progeny in each of the two sets of progeny (giving a maximum θ of 0.50, $z = 0$). Both sets of progeny came from crosses of Scottish *saxatilis*, and this heterogeneity probably arises as a sampling artifact. Twenty-four pairwise locus combinations were tested for heterogeneity in z scores, and it is expected that one of these should yield a significant value. Heterogeneity is, however, a common problem when studying human genetic disease, where apparently identical phenotypes can sometimes be caused by mutations at any one of several loci (Lander, 1988). In our studies, the actual loci under examination have been clearly identified and heterogeneity arising from this source should not therefore be a problem.

Acknowledgements

This work was supported by a research grant to RDW from the Natural Environment Research Council.

References

CAVALLI-SFORZA, L. L. AND BODMER, W. F. 1971. *The Genetics of Human Populations*. Freeman, San Francisco.

JANSON, K. 1983. Chromosome number in two phenotypically distinct populations of *Littorina saxatilis* Olivi from the Swedish west coast. *J. Moll. Stud.*, **49**, 224–227.

JANSON, K. AND WARD, R. D. 1984. Microgeographic variation in allozyme and shell characters in *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae). *Biol. J. Linn. Soc.*, **22**, 289–307.

KNIGHT, A. J. AND WARD, R. D. 1986. Purine nucleoside phosphorylase polymorphism in the genus *Littorina* (Proso-

branchia: Mollusca). *Biochem. Genet.*, **24**, 405–413.

KNIGHT, A. J. AND WARD, R. D. 1990. The genetic relationships of three taxa in the *Littorina saxatilis* species complex (Prosobranchia: Gastropoda). *J. Moll. Stud.* (in press).

LANDER, E. S. 1988. Mapping complex genetic traits in humans. In: Davies, K. E. (ed.) *Genome Analysis: A Practical Approach*, IRL Press, Oxford, pp. 171–189.

MORTON, N. E. 1955. Sequential tests for the detection of linkage. *Am. J. Hum. Genet.*, **7**, 277–318.

MORTON, N. E. 1956. The detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type. *Am. J. Hum. Genet.*, **8**, 80–96.

WARD, R. D. 1990. Biochemical genetic variation in the genus *Littorina* (Prosobranchia: Mollusca). *Hydrobiologia*, **193**, 53–69.

WARD, R. D. AND WARWICK, T. 1980. Genetic differentiation in the molluscan species *Littorina rudis* and *Littorina arcana* (Prosobranchia: Littorinidae). *Biol. J. Linn. Soc.*, **14**, 417–428.

WARD, R. D., WARWICK, T. AND KNIGHT, A. J. 1986. Genetic analysis of ten polymorphic loci in *Littorina saxatilis* (Prosobranchia: Mollusca). *Heredity*, **57**, 233–241.

WARWICK, T. 1983. A method of maintaining and breeding members of the *Littorina saxatilis* (Olivi) species complex. *J. Moll. Stud.*, **48**, 368–370.

WARWICK, T., KNIGHT, A. J. AND WARD, R. D. 1990. Hybridisation in the *Littorina saxatilis* species complex (Prosobranchia: Mollusca). *Hydrobiologia*, **193**, 109–116.