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Environmental stress and allozyme variation in *Littorina littorea* (Prosobranchia)*

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ABSTRACT: The intertidal periwinkle *Littorina littorea*, kept in large experimental basins, was assayed for potential effects of long-term oil exposure on allozyme variation. Genotypic fitness in individuals that successfully settled in oil-exposed water was also examined. Only 2 loci (*6PGDH* and *PGM-2*) of 31 investigated were polymorphic according to the 0.95 criterion of polymorphism. Average expected heterozygosity was estimated at 0.043. The low degree of genetic variation rendered it difficult to spot allozyme markers to be used as indicators of oil stress. Among-sample heterogeneity at the highly polymorphic *6PGDH* locus in adult specimens did not correlate with the presence of oil. There were significant differences between fast growers (but not slow growers) and the initial unexposed population in an oil-exposed basin at the *6PGDH* locus. However, this gave no evidence that fitness is positively correlated with heterozygosity. No significant genetic heterogeneity was found among juveniles in oil-exposed and non-oil-exposed water. Genetic differentiation between pooled samples of juveniles and adults at one low-polymorphic locus *(PGI)* could be attributed to annual variation or developmental selection. In conclusion there is no convincing evidence that *L. littorea* possesses a genetical short-term adaptive strategy to cope with oil contamination.

INTRODUCTION

Like the Norwegian coastline in general, the Oslofjord runs a risk of oil pollution from the growing oil industry and tanker transport. Large land-based experimental basins adjacent to the fjord have been used to study the effect of oil on various aspects of marine life, one of which is the suggestion made by Battaglia et al. (1980) and Lavie et al. (1984) that oil pollution may cause selection for specific genotypes or alleles in populations of marine animals.

The use of littoriniids in studies of the possible correlation between environmental variability, natural or man-made, and genetic polymorphism is not extensive (but see Beardmore & Morris 1978, Wilkins et al. 1978, Janson & Ward 1984, Nevo et al. 1986). However, larval dispersal capacity in various species of littoriniids has been more or less successfully correlated to differences in genetic heterogeneity (e.g. Snyder & Gooch 1973, Berger 1977, Ward & Warwick 1980, Wilkins & O'Regan 1980, Janson 1987). The mid-intertidal periwinkle *Littorina littorea* has a long larval stage (3 to 4 wk) and can therefore disperse widely. One would expect little genetic variation among communities of *L. littorea* sampled in a restricted area and recruited from the same parental population, provided that external selective forces, if present, act in the same way across the sampling sites. When, on the other hand, environmental conditions vary across the area in question, genetic heterogeneity may evolve – if a genetic strategy of adaptation exists.

Because of its reproductive mode, and high abundance in the basins, *Littorina littorea* was selected as one of 3 species to be tested for potential effects of oil contamination on the allozymic variation at specific gene-loci. The other 2 were *Mytilus edulis* (Fevolden & Garner 1986) and *Balanus balanoides* (results not yet published).

We planned to study the potential selective role of oil on *Littorina littorea* in both adult and juvenile communities. Among adults we looked for differences in allelic frequencies between individuals that survived long-term exposure of oil and the initial population. Among juveniles we surveyed differences in genetic composition between those specimens that successfully

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settled in oil-exposed basins and those that settled in control basins or on the adjacent shore. We also hoped to show whether there was a correlation between growth rate and genetic configuration among oil-exposed specimens.

MATERIAL AND METHODS

Basins. The work was done at Solbergstrand Experimental Station on the east coast of the Oslofjord 50 km south of Oslo. We used 4 concrete basins, each measuring 8.0 × 5.0 m, filled with about 25 m^3 of running seawater supplied by centrifugal pumps from an intake at 1 m depth offshore from the basins. Each basin is equipped with a wave generator and tidal simulation reflecting the tidal range and timing of the fjord.

Rocky shore communities had been established in the basins through transplantation of shoreline rocks with algae and animals followed by 3 yr of external recruitment and self propagation before the oil-dosing started in 1982. For the 2 yr from September 1982 to September 1984, 2 of the basins, termed HO and LO (high oil and low oil), were exposed to an average of 129 and 31 µg l^{-1} total hydrocarbons, respectively. Water-accommodated fractions of diesel oil were mixed continuously into the seawater entering the basins. The 2 remaining basins, C2 and C4, were used as controls (see Fevolden & Garner 1986 for more details).

Samples. A total of 15 different samples of *Littorina littorea* were analysed electrophoretically. They were as follows:

B182a, B282a, B382a, B482a: Adults sampled from the 4 basins in September 1982 prior to oil dosing.

FJ82a: Adult controls from the shore immediately outside Solbergstrand sampled in September 1982.

HO83a: Adults from the high oil basin (HO = B1 prior to oil dosing) collected in September 1983, 1 yr after oil dosing started.

HO83j, C283j, LO83j, C483j: New recruits (or juveniles) in the different basins sampled in September 1983, 1 yr after oil dosing started.

FJ83j: New recruits from the fjord control station sampled in September 1983.

HO84a, C284a, LO84a, C484a: Adults sampled in autumn 1984 after 2 yr of oil dosing.

A minimum of 96 individuals from each sample were collected for electrophoretic assay of adults, but the number of specimens which could be reliably scored for individual enzymes was often lower. There were very few new recruits in the basins throughout the experiment. Since this applied to all 4 basins it cannot have been caused by oil-dosing alone, but was probably a result of predation by *Carcinus maenas* (Lystad & Moe 1985). Juveniles were therefore sampled only in autumn 1983. Due to the difficulties in distinguishing between such small individuals of *Littorina littorea* and *L. saxatilis* the number of small juveniles that could be scored was further reduced. The gel runs revealed that some of the juveniles sampled were actually *L. saxatilis*.

Electrophoretic procedures. The electrophoretic procedures used were identical to those referred to in Fevolden & Garner (1986) for *Mytilus edulis*. Whole specimens (except the intestines) were homogenized and used in the starch gel electrophoresis. Five buffer systems were used, referred to in Table 1 as A, B, C, D (as in Fevolden & Garner 1986) and I (Buffer I of Shaw & Prasad 1970).

Genetic variation was determined by measuring frequencies of the different alleles occurring at each locus, frequencies of the different genotypes and by estimating values for observed and expected heterozygosities (according to the Hardy-Weinberg equilibrium).

The genetic nomenclature is similar to that used by Fevolden & Garner (1986). Our allelic designation involves ranking mobilities relative to the commonest allele, which is a different approach from protocols suggesting the use of measured relative mobilities. However, the latter invariably depend on the texture of the gel and it seems hard to obtain measured relative mobilities that do not vary. We have, therefore, found it more appropriate to maintain the use of ranking mobilities.

In statistical analyses where χ^2 values were obtained, rare alleles (expected numbers less than 5) were pooled with neighbouring alleles to check for misinterpretations due to low expected numbers of certain classes (see e.g. Sokal & Rohlf 1969).

RESULTS

Twenty-three enzyme systems plus one unidentified protein were successfully resolved in the initial electrophoretic testing of *Littorina littorea* from the 4 basins prior to oil dosing (Table 1). Thirty loci were scored in adult individuals sampled in 1982 and 1983, but only one, the dimeric *6PGDH,* proved to be polymorphic according to the 0.95 criterion for polymorphism (frequency of the commonest allele should be less than 0.95). Since there is only a small probability of revealing statistically significant among-sample heterogeneity at loci with little allelic variation, it was decided to analyse the remaining samples only for the following enzymes: APH, G3PDH, LAP, 6PGDH, PGI, and PGM. Two *PGM*-loci were resolved. *PGM-2* could only be reliably scored for the last 4 samples, which were analysed after a different buffer system (D) had been

Table 1. Proteins assayed and buffer systems used in the study of *Littorina littorea* from Solbergstrand, Oslofjord. Buffer systems A, B, C & D are described in Fevolden & Garner (1986), and I in Shaw & Prasad (1970)

Protein	Abbreviation	Buffer system
Acid phosphatase	ACPH	A
Aldehyde oxidase	AO	D
Aldolase	ALD	B
Alkaline phosphatase	APH	B (A)
Esterase	EST	A
Fumarase	FUM	B
Glutamate oxaloacetate transaminase (= Aspartate aminotransferase)	GOT	B
Glucose-6-phosphate dehydrogenase	G6PDH	B
Glyceraldehyde-3-phosphate dehydrogenase	G3PDH	B (C, I)
Hexokinase	HK	C
Hydroxybutyrate dehydrogenase	HBDH	B
Isocitrate dehydrogenase	IDH	D
Lactate dehydrogenase	LDH	B
Leucine aminopeptidase	LAP	D (I, A)
Malate dehydrogenase	MDH	B
Malic enzyme	ME	D
Mannose phosphate isomerase	MPI	B
Octanol dehydrogenase	ODH	B
6-Phosphogluconate dehydrogenase	6PGDH	C (B)
Phosphoglucose isomerase	PGI	D (A, B, I)
Phosphoglucomutase	PGM	D (C)
Sorbitol dehydrogenase	SDH	B
Xanthine dehydrogenase	XDH	B
Unidentified protein	PROT	A

taken into use to resolve this enzyme. *PGM-2* turned out to be the second locus that was polymorphic according to the 0.95 criterion. To our knowledge this highly polymorphic *PGM-2* locus has not been resolved in *L. littorea* by other investigators.

Overall heterozygosity and among-sample heterogeneity

Since there was very little genetic variation at most loci, within and between samples, the space-consuming tables with data for all loci in each individual sample have been omitted. Instead, for all loci allelic frequency data have been pooled across the number of samples analysed to give a general impression of the overall genetic variation of the specimens studied (Table 2). Data for individual samples are given only for the 2 loci that were polymorphic according to the 0.95 criterion (Tables 3 & 4). The average observed heterozygosity over the 31 loci was estimated at 0.042 ($\pm$ 0.024), while the expected value was 0.043 ($\pm$ 0.024).

Comparing all samples in a contingency chi square analysis revealed that no locus showed significant among-sample heterogeneity ($p > 0.05$). The most pronounced, although not significant, among-sample heterogeneity was found at the *6PGDH* locus ($\chi^2_{14} = 20.354$, $p > 0.05$), but the variation did not correlate with the presence of oil. This is because the locus revealed significant among-sample heterogeneity in the basins prior to oil dosing ($\chi^2_3 = 17.62$, $p < 0.001$) while after 2 yr of oil exposure this inter-basin heterogeneity had vanished ($\chi^2_3 = 0.47$, $p > 0.9$). None of the remaining 29 loci showed significant among-basin heterogeneity prior to oil-dosing (p single locus > 0.23). Nor did the 6 polymorphic loci (0.99 criterion, Table 2) other than *6PGDH* show heterogeneity in allelic frequencies among the 4 test basins after 2 of the basins had been exposed to oil for 2 yr (p single locus > 0.05).

Size distribution and gene variation

The size distribution of individuals, measured by shell height, showed that although all 4 basins were recruited at the same time, conditions for growth seem to have differed among the basins. The mean size of *Littorina littorea* in Basin C4 was smaller than in the remaining basins at the start of the experiment (Fig. 1). The contemporary significant heterogeneity in allele frequencies at the *6PGDH* locus among the basins might have given evidence of a correlation to growth since the smaller individuals in Sample B482a (as compared to the other basins) had higher frequencies of the most common allele than the remaining basins (Table 3). Two yr later, however, individuals in C4 (C484a) were still smaller than in the 3 other basins (Fig. 1), whereas a correspondingly higher frequency of the most common allele at the *6PGDH* locus was not found (Table 3). The *Littorina* community in this basin grew more slowly than the populations in the other basins probably due to the denser population (density data in Lystad & Moe 1985) and intraspecific competition for available food.

The average size of the fjord control specimens (FJ82a) was similar to the size group found in B482a, that is they were smaller than in 3 of the basins (Fig. 1). Provided the age distribution in basins and fjord was similar this could indicate that the basins in general had a positive effect on growth as compared to the more exposed fjord station.

Table 2. *Littorina littorea*. Allelic frequencies at 31 loci for all samples pooled. N: number of individuals scored; n: number of samples included. H_{obs}: observed frequency of heterozygotes; H_{exp}: expected frequency of heterozygotes; + and –: polymorphic and non-polymorphic loci after the 2 criteria for polymorphism (frequency of the most common allele less than 0.990 and 0.950, respectively)

Locus	N	n	96	98	100	102	104	106	H_{obs}	H_{exp}	$P_{0.990}$	$P_{0.950}$
ACPH	273	5		0.002	0.998				0.003	0.003	–	–
AO-1	141	5		0.004	0.996				0.007	0.007	–	–
ALD-2	338	5			1.000				....	..	–	–
APH	1094	15			0.995	0.005			0.011	0.011	–	–
EST-1	421	5			1.000				...	. .	–	–
EST-2	402	5			0.999	0.001			0.002	0.002	–	–
EST-4	400	5			1.000				..	....	–	–
FUM	314	5			1.000				.. .	...	–	–
GOT	366	5		0.001	0.999				0.003	0.002	–	–
G3PDH	904	14		0.003	0.994	0.003			0.012	0.012	–	–
G6PDH-1	372	5		0.002	0.998				0.005	0.005	–	–
G6PDH-2	241	5			1.000				.	.	–	–
HK-2	346	5			0.996	0.004			0.009	0.009	–	–
HK-3	357	5			0.992	0.008			0.016	0.015	–	–
HBDH-1	264	5			1.000				...	...	–	–
IDH	391	5		0.003	0.997				0.005	0.005	–	–
LDH-2	248	5			1.000				.	. .	–	–
LAP-2	762	11		0.016	0.982	0.001			0.035	0.035	+	–
LAP-3	989	15		0.009	0.989	0.003			0.023	0.022	+	–
MDH-2	439	6			0.999	0.001			0.002	0.002	–	–
ME-2	165	5			1.000				.. .	.	–	–
MPI	405	5			0.998	0.002			0.005	0.004	–	–
ODH	377	5			1.000				...	.	–	–
6PGDH	1115	15		0.003	0.565	0.417	0.015		0.536	0.507	+	+
PGI	1103	15		0.005	0.986	0.009			0.028	0.028	+	–
PGM-1	1117	15	0.002	0.002	0.987	0.009			0.026	0.026	+	–
PGM-2	265	4		0.019	0.533	0.197	0.249	0.002	0.543	0.603	+	+
SDH-1	342	5		0.006	0.988	0.006			0.020	0.020	+	–
XDH	398	5			0.999	0.001			0.002	0.002	–	–
PROT-1	381	5			1.000				..	..	–	–
PROT-4	253	5			1.000					.	–	–

Table 3. *Littorina littorea*. Genetic variation at the *6PGDH* locus N: number of indivudals scored in the different samples. H_{obs}: observed frequency of heterozygotes; H_{exp}: expected frequency of heterozygotes. For sample designation see 'Material and Methods'

Sample	N	98	100	102	104	H_{obs}	H_{exp}
B182a	71	0.000	0.451	0.528	0.021	0.535	0.521
B282a	91	0.000	0.527	0.462	0.011	0.538	0.511
B382a	89	0.006	0.629	0.360	0.006	0.416	0.477
B482a	72	0.000	0.667	0.333	0.000	0.500	0.448
FJ82a	100	0.020	0.510	0.460	0.010	0.520	0.530
HO83a	110	0.000	0.564	0.427	0.009	0.536	0.502
HO83j	17	0.000	0.588	0.382	0.029	0.471	0.522
C283j	37	0.000	0.568	0.405	0.027	0.622	0.520
LO83j	17	0.000	0.559	0.412	0.029	0.412	0.533
C483j	31	0.000	0.597	0.355	0.048	0.516	0.524
FJ83j	86	0.000	0.587	0.413	0.000	0.430	0.488
HO84a	109	0.005	0.569	0.413	0.014	0.596	0.508
C284a	93	0.000	0.586	0.387	0.027	0.495	0.509
LO84a	96	0.000	0.552	0.438	0.010	0.646	0.506
C484a	96	0.000	0.563	0.401	0.036	0.656	0.524

Table 4. *Littorina littorea*. Genetic variation at the *PGM-2* locus after 2 yr of oil-exposure in 2 of the basins. N: number of individuals scored in the different samples; H_{obs}: observed frequency of heterozygotes; H_{exp}: expected frequency of heterozygotes. For sample designation see 'Material and Methods'

Sample	N	98	100	102	104	106	H_{obs}	H_{exp}
HO84a	66	0.030	0.523	0.212	0.227	0.008	0.576	0.634
C284a	48	0.010	0.542	0.146	0.302	0.000	0.500	0.600
LO84a	75	0.013	0.513	0.227	0.247	0.000	0.600	0.628
C484a	80	0.019	0.556	0.188	0.238	0.000	0.563	0.602

Slow and fast growing oil-exposed individuals

After 1 yr of oil exposure in HO the allelic frequencies in 2 categories of individuals were compared. The tagged specimens (Sample HO83a) were categorized either as fast growers (annual shell height increase

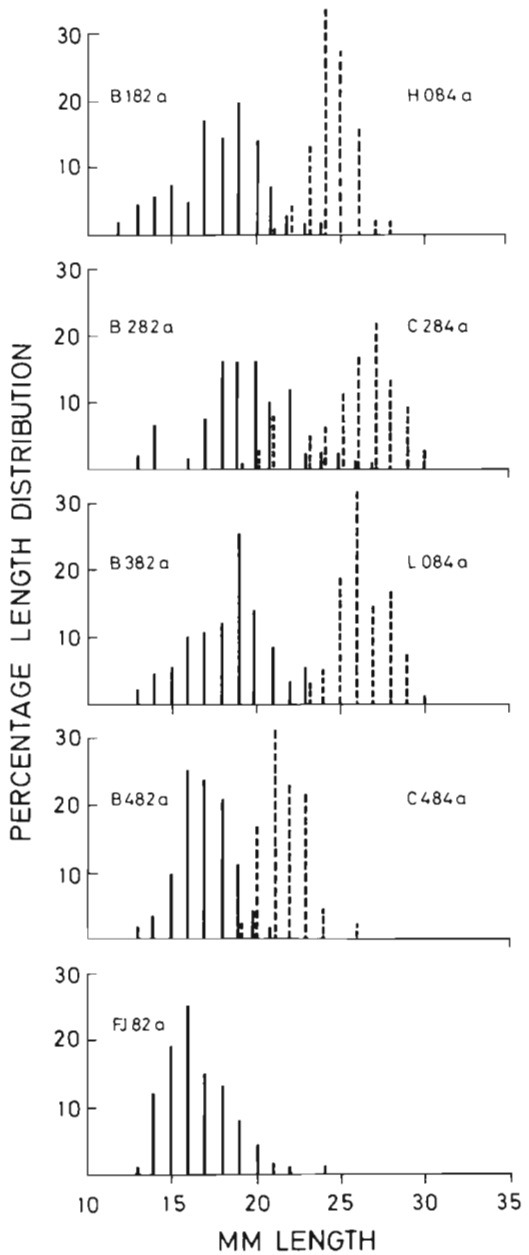

Fig. 1. *Littorina littorea*. Length distribution of different samples

>4 mm) or slow growers (annual shell height increase <2 mm). Chi-square contingency tests of the 30 loci analysed showed no significant heterogeneity between the 2 groups at any locus (p single locus >0.08, additive p for all polymorphic loci = 0.283). Again, the difference in allele frequencies at the *6PGDH* locus between the 2 groups is pronounced (Table 5), but not statistically significant (Table 6). However, when fast growers in HO are compared to the original population in B1 (HO prior to oil dosing), a significant change in allele frequencies at *6PGDH* is observed, whereas this is not the case when slow growers are compared to the original population (Table 6). The frequency of rare alleles was lowered among fast growers (Table 5).

Table 5. *Littorina littorea*. Allelic frequencies at the *6PGDH* locus for fast and slow growing individuals in Sample HO83a after 1 yr of oil exposure. Data for the initial population in that basin is included for easy comparison. N: number of individuals scored; H_{obs}: observed frequency of heterozygotes; H_{exp}: expected frequency of heterozygotes

	N	100	102	104	H_{obs}	H_{exp}
Fast	63	0.611	0.381	0.008	0.524	0.485
Slow	47	0.500	0.489	0.011	0.553	0.516
B182a	71	0.451	0.528	0.021	0.535	0.521

Inter-basin comparison of juveniles; comparison of juveniles with adults, and of basins with fjord

The juveniles exhibited no significant differences in allele frequencies between oil basins and non-oil basins at any locus (p single locus >0.16, p total = 0.89). When the data for recruits in all 4 basins were pooled and compared to fjord recruits, contingency table analyses revealed no significant differences (p single locus >0.1, p total >0.7).

A comparison of the pooled group of all juvenile samples and the pooled adult samples reveals significant differences at one locus, *PGI* (χ^2 = 7.351, p <0.01, rare alleles pooled). When the significance level is adjusted to the number of tests, in this case number of polymorphic loci compared (= 5), to correct for the I-error (Cooper 1968), a p significance level of 0.05/5 = 0.01 must be used. The difference at the *PGI* locus is significant after this correction of the significance level and is caused by higher frequencies of rare alleles among juveniles than among adults. This is not surprising considering the possibilities of yearly variation and also the probability that juveniles and adults have different metabolic requirements. Selection may occur from juvenile stages to adults because of different fitness of the offspring.

Differences in allele frequencies between the adult

Table 6. *Littorina littorea*. Chi-square test of heterogeneity at the *6PGDH* locus when fast and slow growing individuals in Sample HO83a are compared to the initial population in the same basin (B182a). Degrees of freedom = 1

	Fast	B182a
Slow	χ^2 = 2.70, p > 0.05 ns	χ^2 = 0.55, p > 0.8 ns
Fast		χ^2 = 6.89, p < 0.01

fjord controls (FJ82) and the pooled sample of pre-oil basin adults were all within the limits of sampling error ($p > 0.05$).

Deviations from Hardy-Weinberg equilibrium

Chi-square tests and F-statistics (Wright 1965, 1978) were used to test for deviations from Hardy-Weinberg expectations in a randomly mating population. *6PGDH* was the only locus of the 31 that at any time showed significant deviations from Hardy-Weinberg equilibrium. All pre-oil basin populations had *6PGDH* genotype frequencies according to expected values, so had adults in HO after 1 yr of oil exposure ($p > 0.480$). However, 2 yr after the experiment started, 3 of 4 basins showed heterozygous overdominance at the *6PGDH* locus (2 statistically significant; Table 7). The fact that a significant surplus of heterozygotes was seen in both oil and non-oil basins gave evidence that the oil alone did not provoke selection for heterozygotes.

Table 7. *Littorina littorea*. Chi-square values and F(IS) values (Wright 1965, 1978) for deviations from Hardy-Weinberg equilibrium for *6PGDH* genotypes in the 4 basins at the termination of the experiment. Significance of F(IS) was measured according to Li (1955); $\chi^2 = NF^2(IS)$ (N = sample size). Degrees of freedom = 1

Sample	χ^2	p	F(IS)	χ^2	p
HO84a	2.619	0.106 ns	−0.179	3.492	>0.05 ns
C284a	0.256	0.613 ns	0.023	0.049	>0.8 ns
LO84a	8.677	0.003	−0.282	7.634	<0.01
C484a	9.047	0.003	−0.259	6.440	<0.02

DISCUSSION

The average heterozygosity of the *Littorina littorea* population analysed here is low and does not fit the expectation of higher heterozygosity for mollusc species with high dispersal of young (see discussion in Lavie & Nevo 1986). The value of $\bar{H}$ was actually increased from 0.008 to 0.042 by the contribution from 2 loci, *6PGDH,* and *PGM-2*. Multi-loci analyses of this species from other areas are very few. Two studies (Morris 1979, Janson 1987) confirm the relatively low degree of genetic variation. Morris measured the average expected frequency of heterozygotes over 24 loci to be 0.097 (British Isles) while Janson's estimate over 16 loci was 0.037 (Swedish west coast). Both investigators, however, reported more pronounced variation at specific loci (e.g. *PGI*) than in the present investigation. This low degree of genetic variation in *L. littorea* in the Oslofjord rendered it difficult to test our initial hypothesis that exposure to low concentrations of oil should alter the frequencies of alleles and genotypes. It has been acknowledged (e.g. Nevo et al. 1986) that the higher the heterozygosity of a species the greater the probability that it would provide a good genetic monitor for pollution.

The basin populations of *Littorina littorea* showed divergence in allelic frequencies at the *6PGDH* locus before oil-exposure started despite the fact that all 4 basins were recruited at the same time from the same water and thus the same parental population and cohort of larvae. Between 71 and 91 specimens were scored for *6PGDH* in the 4 pre-oil basins. Assuming that this allows a reasonable level of statistical precision in the heterogeneity tests, the reason for the pre-oil heterogeneity at this locus may have been differential selection in the basins.

It was shown that the *6PGDH* variation could not be correlated to observed differences in growth among the basins. Also intra-basin differences in growth rate gave no evidence of correlation between growth and heterozygosity (Table 5). Thus our data do not support the hypothesis of enhanced heterozygosity among fast growers (see Singh & Zouros 1978, Zouros et al. 1980, and discussion in Mallet et al. 1986). However, survival alone in the basins over a prolonged period may be related to heterozygous overdominance at certain loci as indicated by the heterozygous surplus of *6PGDH* at the end of the experiment (Table 7; F[IS] is negative).

Moreover, although there might be a genetic component to fitness in the oil-exposed basins, the 2 highly polymorphic loci in *Littorina littorea, 6PGDH* and *PGM-2,* either respond indifferently to the presence of oil, or the response is distorted by uncontrolled variables causing synergistic or antagonistic effects. This also applies to a potential basin effect, independent of the presence of oil, since the heterozygous overdominance at *6PGDH* in the basin populations of *L. littorea* in autumn 1984 was accompanied by a deficit of heterozygotes (statistically insignificant) at the *PGM-2* locus (Table 4).

Unlike *Mytilus edulis* in the basins (Fevolden & Garner 1986), adult *Littorina littorea* seemed to have sufficient adaptive capacitiy to cope with the new environment. The viability of adults did not seem to be dramatically affected in 3 of the 4 basins. In the HO basin the decrease in the population size of *L. littorina* over the 2 yr of the experiment was significantly higher than in the other basins (Moe & Lystad unpubl.). However, this effect should be compared to the total extinction of *M. edulis* in the same basin over the same period (Fevolden & Garner 1986).

The adaptive capacity, or fitness, of juveniles is

difficult to assess since the number of juveniles was low in all 4 basins. The lowest number of recruits, however, was found in the oil basins (Lystad & Moe 1985) and is likely to be correlated to toxic effects of the oil. No statistically significant correlation to any of the genetic components analysed was found.

Lavie & Nevo (1982) studied heavy metal selection of *PGI* allozymes in 2 different gastropod species, *Monodonta turbinata* and *M. turbiformis*, and concluded that their results were inconsistent with the neutral theory of allozyme polymorphism (Kimura 1968). Instead they suggested that their results reflected an adaptive strategy of specific genotypes. This hypothesis gained further support from Nevo et al. (1983) who concluded that different allelic isozyme genotypes of the 2 *Monodonta* species and of the shrimp *Palaemon elegans* react differently to the quality and quantity of specific pollutants. More recently, Nevo and coworkers (1986) have presented evidence that within families of marine gastropods, including *Littorina*, species with a higher level of genetic diversity are more resistant to pollutants than species with low genetic diversity. *Littorina saxatilis*, which is genetically much more variable than *L. littorea* (Janson 1987), was never permanently established in the basins despite its occurrence on the nearby shore. Juveniles were observed in low numbers in all basins (see 'Material and Methods'), adults only in one control basin. It was therefore impossible to clarify whether *L. saxatilis* is more resistant to oil-pollution than *L. littorea*, as suggested by the hypothesis of Nevo et al. (1986).

The genetic impact of oil pollution has been studied by Battaglia et al. (1980; *Tisbe holothuriae* and *Mytilus galloprovincialis*) and Lavie et al. (1984; *Monodonta turbinata* and *M. turbiformis*). Both studies suggested that oil pollution caused a decrease in the frequency of heterozygotes at specific loci. Significant differences in viability were found, especially for genotypes of *PGI*. Whilst the present data alone are not sufficient to reject the role of pollutants in adaptive selection, parallel studies of *Mytilus edulis* (Fevolden & Garner 1986) and *Balanus balanoides* (Sigurdsson & Fevolden unpubl.) do not support the findings of Battaglia et al. (1980) and Lavie et al. (1984). We suggest, therefore, that major groups of marine invertebrates do not possess a genetical adaptive strategy to oil pollution. Viability under such environmental stress for those animals may be a mere phenotypic phenomenon – or related to genetical characters other than the allozymes being studied.

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