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Size-related and geographic variation at two enzyme loci in *Megalorchestia californiana* (Amphipoda: Talitridae)

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Genetic variation in the enzymes mannose phosphate isomerase and glucosephosphate isomerase was surveyed in the amphipod *Megalorchestia californiana* Brandt on the Pacific coast of the United States. In a series of samples from Washington state to southern California, both enzyme loci exhibited differences of allele frequency associated with latitude. Geographic differences among nine Oregon samples were not consistently associated with any obvious environmental variable.

Within the Oregon samples, the *Mpi* locus exhibited a heterozygote deficit in the smallest size class. Larger size classes did not differ from Hardy-Weinberg proportions. Possible explanations for this pattern include genotype-related differences in behavior, growth or viability.

INTRODUCTION

Several types of evidence from natural populations have been used to infer natural selection on enzyme loci. These include associations of allele frequency with environment; deviations from Hardy–Weinberg proportions; differences in allele frequency between sexes or among size or age classes; and temporal changes in allele frequency. Here I examine the loci coding two polymorphic enzymes, mannose phosphate isomerase (MPI, E.C. 5.3.1.8) and glucosephosphate isomerase (GPI, E.C. 5.3.1.9), in the talitrid amphipod *Megalorchestia californiana* Brandt (formerly *Orchestoidea californiana*; see Bousfield, 1982).

This species is an appropriate subject for ecological genetics. Individuals remain in burrows or nestled under debris on sand beaches during the day, emerging at night to feed (Bowers, 1964). Because foraging movements are wide ranging and individuals do not return to particular burrows, the amphipods on a beach are a single well mixed population. Juveniles and adults are weak swimmers and avoid water; compared to marine animals with planktonic larvae, there is little dispersal among beaches separated by rocky headlands, river mouths and other barriers. Hundreds of *M. californiana* are present per linear meter of beach (Bowers, 1964), making large samples possible. In California, breeding occurs from early spring through late autumn (McClurkin, 1953; Bowers, 1964).

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MATERIALS AND METHODS

Amphipods were captured at night with pitfall traps, consisting of plastic cups buried flush with the sand and containing 2 to 3 cm of seawater. Amphipods were maintained at 3°C on moist paper towels. Mortality during capture, return to the laboratory and storage of up to several weeks was negligible. MPI and GPI phenotypes did not differ between freshly caught amphipods and those which had been maintained in the laboratory for several weeks.

The locations sampled are shown in figs. 1 and 2, and sampling dates are listed in table 2(a). The live weight of each individual in the Oregon samples was recorded. The sex of each individual weighing 0·2 g or more was determined using the enlarged second gnathopod of males; the sex of smaller individuals could not be determined easily.

Vertical polyacrylamide gel electrophoresis was used; gels contained 7 per cent acrylamide monomer and 0·35 per cent bisacrylamide. Gels

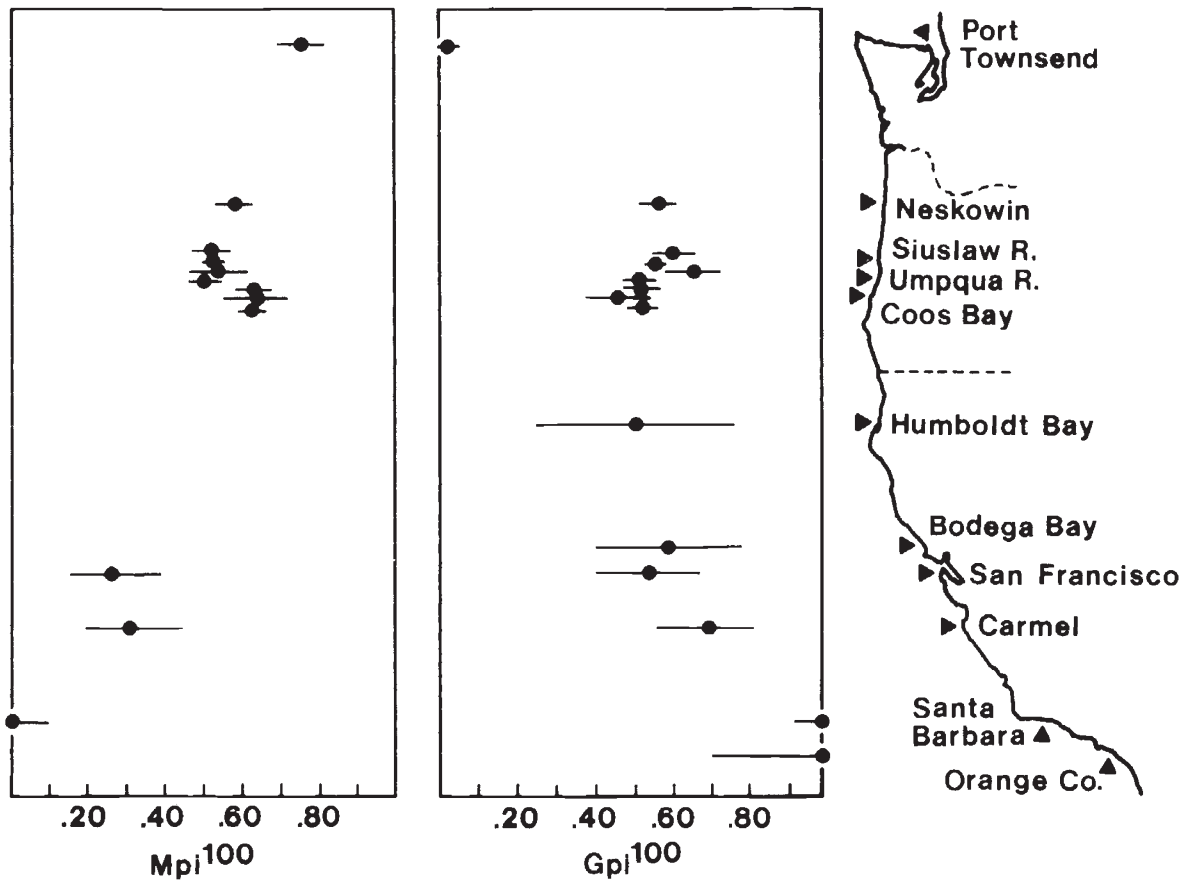

Figure 1 Mpi^{100} and Gpi^{100} allele frequencies on the Pacific coast of the United States. Horizontal bars represent 95 per cent confidence intervals.

were run at 3°C with 4 mA of current for 30 minutes followed by 25 mA for 3 hours. MPI was run on the continuous tris-borate buffer of Ayala *et al.* (1973) and was detected by the method of Nichols and Ruddle (1973). GPI was run on Poulik's buffer system (Poulik, 1957) and was detected by the method of Shaw and Prasad (1970).

A single leg (or several legs from the smaller individuals) was removed from each amphipod and macerated in about 40 μl of distilled water containing 5 per cent sucrose. Each amphipod was then maintained alive for up to a week in a vial with a scrap of moistened paper towel. Another leg was used if there was any ambiguity about the scoring of the MPI or GPI phenotype. For MPI, 5 to 10 μl of crude homogenate were loaded into each sample well of the gel, and 1 to 2 μl samples were used for GPI.

Deviations from Hardy-Weinberg genotype proportions were examined using Wright's inbreeding coefficient $F_{IS} = (H_e - H_o)/H_e$, where H_e is the expected number of heterozygotes, calculated using Levene's (1949) correction for small sample size, and H_o is the observed number of heterozygotes. A positive F_{IS} indicates a deficit of heterozygotes relative to Hardy-Weiknberg expectations, and a negative F_{IS} indicates an excess. $\bar{F}_{IS}$, the weighted mean inbreeding coefficient, was calculated using equation (6) of Kirby (1975) for each size class and for the total population samples. This equation takes into account differences in allele frequency among populations in calculating the mean F_{IS}. When calculating $\bar{F}_{IS}$ of size classes, only subsamples with 10 or more individuals were used. Statistical significance was tested using $d^2 = F_{IS}{}^2X$, where X is the number of individuals in the sample; d^2 is chi-square distributed (Christiansen *et al.*, 1976). The criterion of Cooper (1968) for simultaneous tests was used: to be significant at the 5 per cent

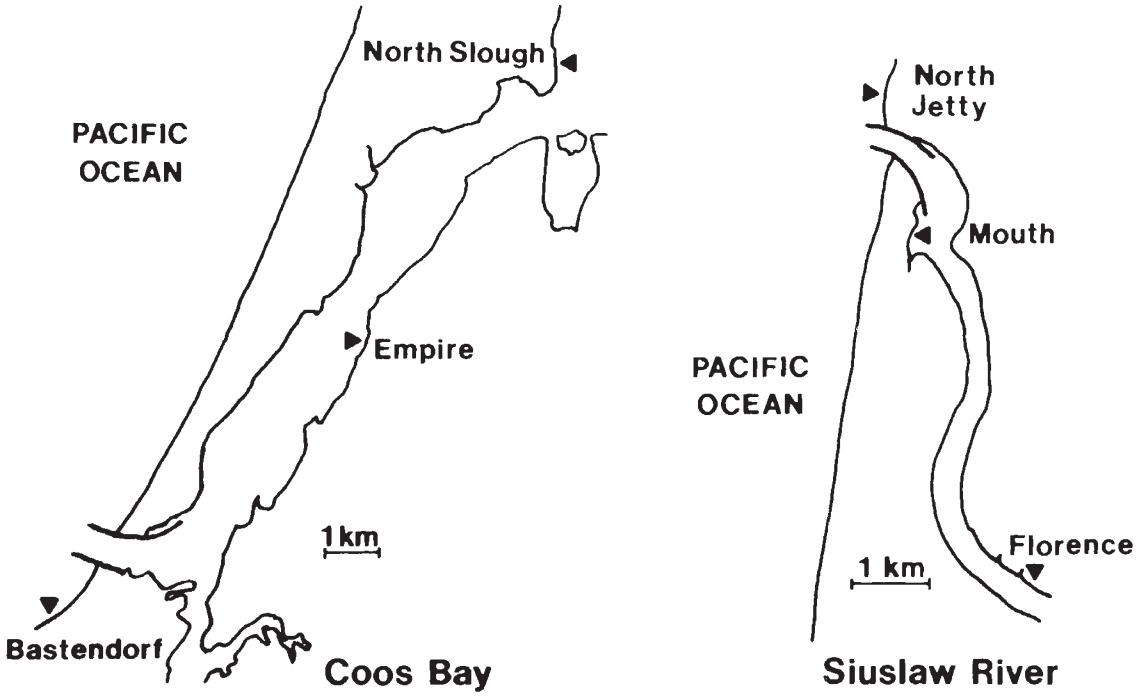

Figure 2 Sample sites at Siuslaw River and Coos Bay, Oregon.

level, one test must have an individual probability less than 0·05 divided by the number of tests.

Variation in allele frequency among size classes was examined by determining the difference in allele frequency between each size class and the remaining size classes. The weighted mean of these deviates was determined over all the population samples. Significance was tested using the method of Cochran (1954; Snedecor and Cochran, 1980, pp. 210–213) with the continuity correction of Mantel and Haenszel (1959). This method was also used to compare allele frequencies of males and

Table 1 *Mpi* and *Gpi* allele frequencies. *N*, number of individuals. —, no data. *, $N = 40$ for *Mpi*

		Mpi					*Gpi*				
Location	*N*	85	90	96	100	105	null	90	95	100	105
Port Townsend	93		0·253		0·747				0·978	0·022	
Neskowin	209	0·005	0·419	0·002	0·574				0·433	0·567	
Siuslaw-N. Jetty	162		0·481		0·519			0·003	0·392	0·605	
Siuslaw-Mouth											
28 Feb.	638		0·479		0·521			0·013	0·418	0·567	0·002
15 Aug.	255		0·484		0·516		0·002	0·004	0·443	0·551	
total	893		0·480		0·520		0·001	0·011	0·426	0·562	0·001
Siuslaw-Florence	80		0·462		0·538			0·013	0·325	0·663	
Umpqua	238	0·002	0·498		0·500			0·008	0·473	0·519	
Coos-Bastendorf	289	0·002	0·377		0·619	0·002		0·005	0·466	0·527	0·002
Coos-Empire	67		0·366		0·627	0·007			0·537	0·463	
Coos-N. Slough	183		0·372		0·626	0·003			0·475	0·525	
Humboldt Bay	8		—		—				0·500	0·500	
Bodega Bay	12		—		—				0·417	0·583	
San Francisco	27	0·037	0·704		0·259			0·019	0·444	0·537	
Carmel	28	0·196	0·500		0·304				0·304	0·696	
Santa Barbara	80*		1·00							0·769	0·231
Orange County	4		—		—					1·00	

females. *G*-tests were used to test for heterogeneity of allele frequency among samples (Sokal and Rohlf, 1981).

RESULTS

MPI phenotypes were either one- or two-banded in both males and females, and GPI phenotypes were either one- or three-banded in both males and females. This is consistent with MPI being a monomer and GPI a dimer, both coded by autosomal loci with codominant alleles, as is the case in other amphipods (Busath, 1980; Bulnheim and Scholl, 1981) and in many other crustaceans (Hedgecock *et al.*, 1982). The common allozyme which migrated furthest towards the anode was designated 100, and other allozymes were named by adding to or subtracting from 100 the mobility difference in millimetres. The genetic loci coding for the enzymes MPI and GPI are designated *Mpi* and *Gpi*. For statistical purposes, rare alleles were

Table 2 Inbreeding coefficients, F_{IS}, of the nine Oregon samples. $\bar{F}_{IS}$, weighted mean F_{IS}. *N*, number of individuals. —, F_{IS} not calculated

(a) F_{IS} of *Mpi*. Under each size class are listed the number of $Mpi^{90/90}$, $Mpi^{90/100}$, $Mpi^{100/100}$, and F_{IS}. All sampling dates are 1984

Location / Sampling dates	Size class, g 0·0–0·1	0·1–0·2	0·2–0·3	0·3–0·4	0·4–0·5	0·5+	total
Neskowin	0	1	3	18	4	7	33
3 July	0	8	27	48	12	16	111
	0	1	15	24	10	15	65
	—	−0·520	−0·278	0·065	0·044	0·130	−0·085
Siuslaw-N. Jetty	5	11	10	9	4	3	42
5 April	12	21	26	9	3	1	72
20 April	13	10	12	8	3	2	48
12 July	0·153	0·011	−0·074	0·320	0·424	—	0·113
Siuslaw-Mouth	15	52	31	27	22	5	152
28 Feb.	18	110	78	51	42	8	307
	12	53	42	39	21	12	179
	0·205	−0·021	−0·035	0·123	0·017	0·319	0·037
Siuslaw-Mouth	21	7	4	14	12	1	59
15 Aug.	26	24	10	42	15	12	129
	14	10	4	21	7	11	67
	0·143	−0·163	−0·080	−0·093	0·111	−0·185	−0·011
Siuslaw-Florence	6	0	6	4	0	0	16
20 April	12	5	16	8	1	0	42
7 May	7	7	4	3	1	0	22
12 July	0·058	−0·211	−0·214	−0·036	—	—	−0·049
Umpqua	15	38	5	2	1	1	62
28 Feb.	21	72	17	3	1	0	114
	21	29	10	1	1	0	62
	0·261	−0·037	−0·072	—	—	—	0·044
Coos-Bastendorf	8	12	10	5	6	3	44
7 May	12	23	39	32	17	8	131
	9	30	30	24	13	8	114
	0·186	0·239	−0·048	−0·152	0·032	0·119	0·039
Coos-Empire	0	1	5	0	2	3	11
6 June	0	2	14	5	4	2	27
	1	4	12	4	6	2	29
	—	—	0·064	—	0·281	—	0·138
Coos-N. Slough	0	5	10	10	2	0	27
18 June	1	9	38	15	11	8	82
	1	9	33	13	10	8	74
	—	0·211	−0·014	0·216	−0·065	−0·292	0·043
$\bar{F}_{IS}$	0·179	−0·007	−0·066	0·021	0·061	0·064	0·026
N	247	547	511	424	226	122	2121

(b) F_{IS} of *Gpi*. Under each size class are listed the number of $Gpi^{95/95}$, $Gpi^{95/100}$, $Gpi^{100/100}$, and F_{IS}

Location	Size class, g 0·0–0·1	0·1–0·2	0·2–0·3	0·3–0·4	0·4–0·5	0·5+	total
Neskowin	0	2	9	13	4	6	34
	0	8	27	46	15	17	113
	0	0	9	31	7	15	62
	—	−0·583	−0·187	−0·059	−0·147	0·065	−0·098
Siuslaw-N. Jetty	6	8	7	2	1	1	25
	12	22	21	12	7	4	78
	12	12	20	12	2	1	59
	0·181	−0·045	0·066	−0·063	−0·343	—	−0·004
Siuslaw-Mouth	4	37	28	23	20	4	116
28 Feb.	29	108	71	54	44	13	319
	12	70	52	40	21	8	203
	−0·316	−0·026	0·038	0·061	−0·029	−0·046	−0·018
Siuslaw-Mouth	8	9	5	13	8	7	50
15 Aug.	34	23	4	37	19	12	129
	19	9	9	27	7	5	76
	−0·143	−0·108	0·545	0·013	−0·102	0·014	−0·020
Siuslaw-Florence	2	0	6	1	0	0	9
	12	5	8	10	1	0	36
	11	7	12	4	1	0	35
	−0·081	−0·211	0·362	−0·343	—	—	0·000
Umpqua	14	29	7	2	1	0	53
	29	77	15	1	1	0	123
	14	33	10	3	1	1	62
	−0·009	−0·105	0·069	—	—	—	−0·033
Coos-Bastendorf	5	13	19	15	8	3	63
	14	37	35	29	19	12	146
	10	15	25	17	9	4	80
	0·022	−0·131	0·114	0·056	−0·042	−0·233	−0·012
Coos-Empire	1	2	13	2	3	1	22
	0	3	12	4	6	3	28
	0	2	6	3	3	3	17
	—	—	0·197	—	0·042	—	0·166
Coos-N. Slough	0	3	23	6	8	3	43
	1	14	36	18	10	9	88
	1	6	22	14	5	4	52
	—	−0·212	0·116	0·022	0·135	−0·094	0·039
F_{IS}	−0·078	−0·087	0·090	0·002	−0·049	−0·037	−0·014
N	247	547	511	424	226	122	2121

pooled with the alleles for the electrophoretically closest common allozymes. One individual exhibited a two-banded GPI phenotype, which could result from a heterozygote of Gpi^{100} and an allele producing an inactive allozyme; the presumed null allele was pooled with Gpi^{95}.

Both the *Mpi* and *Gpi* loci showed geographic differences of allele frequency associated with latitude (table 1, fig. 1). Mpi^{100} and Gpi^{95} were greatest in frequency at Port Townsend, Washington, the northernmost location sampled. These alleles were lowest in frequency at Santa Barbara, California, and were intermediate in frequency in Oregon and northern California.

The *Mpi* allele frequencies in nine Oregon samples were significantly heterogeneous ($G = 19{\cdot}20$, $P < 0{\cdot}01$). The *Gpi* allele frequencies within Oregon were not heterogeneous overall ($G = 9{\cdot}42$, $P > 0{\cdot}10$). The Siuslaw-Mouth sample, from near the mouth of the Siuslaw River estuary (fig. 1), had a significantly lower frequency of the Gpi^{100} allele than the Siuslaw-Florence sample, from 6 km upstream ($G = 6{\cdot}04$, $0{\cdot}025 > P > 0{\cdot}01$). No such difference was present at a second estuary, Coos Bay, where the frequencies of Gpi^{100} were virtually identical outside the estuary, at Coos-Bastendorf, and 14 km upstream, at Coos-North Slough. One location, Siuslaw-Mouth, was sampled in February

and again in August to test for seasonal changes in allele frequency. Neither *Mpi* nor *Gpi* allele frequency differed significantly between the two samples (table 1).

Of the nine Oregon samples, none had an inbreeding coefficient, F_{IS}, which differed significantly from zero at either locus (table 2). However, when the samples were divided into size classes, the 0·0–0·1 g class exhibited a deficit of heterozygotes (positive $\bar{F}_{IS}$) at the *Mpi* locus. Because the forces causing deviations from Hardy–Weinberg proportions would likely affect the two loci differently, the data for each locus were considered a separate set of seven simultaneous tests (the six size classes and the total $\bar{F}_{IS}$). The individual probability for the $\bar{F}_{IS}$ of *Mpi* in the 0·0–0·1 g class was 0·005, which is significant at the 5 per cent level (Cooper, 1968). At the *Gpi* locus, none of the $\bar{F}_{IS}$ values differed significantly from zero.

Neither *Mpi* nor *Gpi* differed significantly in allele frequency between males and females, either in whole population samples or in size classes taken separately. There was no significant size-related variation in allele frequency at either locus.

DISCUSSION

There were differences in allele frquency associated with latitude at both the *Mpi* and *Gpi* loci in *M. californiana*. Latitudinal differences at loci coding for GPI are present in many organisms; there are few reported examples of latitudinal MPI differences, perhaps because MPI is often not included in routine electrophoretic surveys. Selection by differences in temperature or some other environmental variable is often inferred to be the cause of latitudinal differentiation. However, it could also result from random drift in isolated populations followed by migration. On a smaller scale, *Mpi* allele frequencies within Oregon were significantly heterogeneous; the variation was not associated with a known environmental variable.

At the *Mpi* locus there was a significant deficit of heterozygotes in the smallest size class. Heterozygote deficits are common at enzyme loci in marine animals, and the pattern of heterozygote deficit only in small or young individuals is present in the bivalves *Geukensia demissa* (= *Modiolus demissus*) (Koehn *et al.*, 1973), *Mytilus californianus* (Tracey *et al.*, 1975), *Crassostrea virginica* (Zouros *et al.*, 1980), and *Mytilus edulis* (Koehn and Gaffney, 1984) in the fish *Rhombosolea plebia* (Smith and Francis, 1984), and in the terrestrial isopod *Porcellio scaber* (Sassaman, 1978). There are a number of possible explanations for a heterozygote deficit, some of which would also explain its presence in only the smallest size class.

Unscored individuals

Because the phenotype of an *Mpi* heterozygote consists of two bands rather than one, heterozygotes are more likely to be too faint to score. In the 0·0–0·1 g class, five amphipods which could not be reliably scored died or escaped before they could be electrophoresed a second time. Even if all five were heterozygotes, the $\bar{F}_{IS}$ would only be reduced from 0·179 to 0·156.

Mis-scored individuals

A total of 254 individuals were electrophoresed twice and scored both times for *Mpi*. In only one case did the second scoring differ from the first, an error rate whch is far too small to account for the observed heterozygote deficit.

Null allele

To account for the observed $\bar{F}_{IS}$, a null allele would have to have a frequency of at least 0·086 in the smallest size class (Zouros and Krimbas, 1969). The heterozygote deficit is not seen in adults, which implies strong selection against the null allele; the mutation rate then required to recreate the null allele polymorphism in each generation is unrealistically high.

Population mixing

To produce the observed $\bar{F}_{IS}$ of 0·179 by mixing of differentiated populations would require the equal mixing of populations differing in allele frequency by 0·42. This is much greater than the range of *Mpi* allele frequencies found in the eight Oregon locations sampled, which suggests that population mixing is not the cause of the heterozygote deficit. The natural history of *M. californiana* also makes it unlikely: migrants from one beach to another would either have to hop overland for considerable distances or get swept to sea and then deposited on another beach, which suggests that immigrants are probably a small proportion of most populations.

Seasonal mixing

Large seasonal changes in allele frequency, with subsequent mixing of individuals selected on dur-

ing different seasons, are unlikely; at the *Mpi* locus there was no significant difference in allele frequency between February and August.

Cryptic species

Inadvertent sampling of two species, with the two species differing in allele frequency, can also be dismissed. Of the three other *Megalorchestia* species known in Oregon, only *M. benedicti* shares its MPI and GPI allozymes with *M. californiana* (J.H. McDonald, unpublished). *M. benedicti* is monomorphic for Mpi^{90} in Oregon (McDonald 1984). Adult *M. benedicti*, which are easily recognized, were found only at the Siuslaw-North Jetty site, so inadvertent sampling of *M. benedicti* might explain the heterozygote deficit in only one of the six samples where it was found.

Non-random sampling

If heterozygous individuals spent less time hopping about, they would have been less likely to fall into the pitfall traps used in this study. Most of the samples were collected between dusk and midnight; if heterozygotes emerged from their burrows in the early morning they would also be less likely to get sampled. Behavioral differences related to enzyme genotypes have been suggested for the fish species *Gymnocephalus cernua* (Nyman, 1975) and *Fundulus heteroclitus* (DiMichele and Powers, 1982), the isopod *Asellus aquaticus* (Christensen, 1977), and *Colias* butterflies (Watt *et al.*, 1983).

Assortative mating

Assortative mating with respect to the *Mpi* locus could occur if different *Mpi* genotypes bred at different times, or through a combination of size-assortative mating and size-related variation in allele frequency. If assortative mating is the cause of the heterozygote deficit, strong selection favoring heterozygotes would also be required to explain the lack of deficit in the larger size classes.

Selection against heterozygotes

Viability selection against heterozygotes might produce the heterozygote deficit. Selection would have to favour heterozygotes in older amphipods in order to erase the deficit in larger size classes. Reversing selection at different life stages has been postulated to explain heterozygote deficits in bivalves (Singh and Green, 1984; Zouros and Foltz, 1984).

Faster growing heterozygotes

In a number of studies of organisms in which the age of the individuals is known, heterozygotes grow faster than homozygotes (Mitton and Grant, 1984). The amphipods in this study were divided into size, not age, classes. If *Mpi* heterozygotes grow faster than homozygotes, heterozygotes would spend less time in the smallest size class and thus a heterozygote deficit would result.

There are four plausible explanations for the deficiency of *Mpi* heterozygotes in the smallest size class: assortative mating, followed by selection favoring heterozygotes; selection against heterozygotes, followed by selection favoring heterozygotes; non-random sampling caused by genotype related differences in behavior; or genotype related differences in growth rate.

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REFERENCES

AYALA, F. J., HEDGECOCK, D., ZUMWALT, G. S. AND VALENTINE, J. W. 1973. Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution*, *27*, 177–191.

BOUSFIELD, E. L. 1982. The amphipod superfamily Talitroidea in the northeastern Pacific region. I. Family Talitridae: systematics and distributional ecology. *Natn. Mus. Natur. Sci. (Ottawa) Publ. Biol. Oceanogr.*, *11*, 1–73.

BOWERS, D. E. 1964. Natural history of two beach hoppers of the genus *Orchestoidea* (Crustacea: Amphipoda) with reference to their complemental distribution. *Ecology*, *45*, 677–696.

BULNHEIM, H.-P. AND SCHOLL, A. 1981. Electrophoretic approach to the biochemical systematics of gammarids. *Helgolander Meeresunters.*, *34*, 391–400.

BUSATH, A. L. 1980. Genetic differentiation of the semi-terrestrial amphipod *Orchestia traskiana* in an expanded habitat on Santa Cruz Island. In Power, D. M. (ed.) *The California Islands: Proceedings of a multidisciplinary symposium.* Santa Barbara, California, Santa Barbara Museum of Natural History, pp. 395–401.

CHRISTENSEN, B. 1977. Habitat preference among amylase genotypes in *Asellus aquaticus* (Isopoda, Crustacea). *Hereditas*, *87*, 21–26.

CHRISTIANSEN, F. B., FRYDENBERG, O., HJORTH, J. P. AND SIMONSEN, V. 1976. Genetics of *Zoarces* populations. IX. Geographic variation at the three phosphoglucomutase loci. *Hereditas*, *83*, 245–256.

COCHRAN, W. G. 1954. Some methods for strengthening the common chi^2 tests. *Biometrics*, *10*, 417–451.

COOPER, D. W. 1968. The significance level in multiple tests made simultaneously. *Heredity, 23*, 614–617.

DIMICHELE, L. AND POWERS, D. A. 1982. Physiological basis for swimming endurance differences between LDH-B genotypes of *Fundulus heteroclitus*. *Science, 216*, 1014–1016.

HEDGECOCK, D., TRACEY, M. L. AND NELSON, K. 1982. Genetics. In Abele, L. G. (ed.) *The biology of crustacea, Vol. 2: Embryology, morphology, and genetics*. New York, Academic Press, pp. 283–403.

KIRBY, G. C. 1975. Heterozygote frequencies in small subpopulations. *Theor. Popul. Biol., 8*, 31–48.

KOEHN, R. K., AND GAFFNEY, P. M. 1984. Genetic heterozygosity and growth rate in *Mytilus edulis*. *Mar. Biol., 82*, 1–7.

KOEHN, R. K., TURANO, F. J. AND MITTON, J. B. 1973. Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus*. *Evolution, 27*, 100–105.

LEVENE, H. 1949. On a matching problem arising in genetics. *Ann. Math. Statist., 20*, 91–94.

MANTEL, N. AND HAENSZEL, W. 1959. Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natn. Cancer Inst., 22*, 719–748.

MCCLURKIN, J. I. JR. 1953. *Studies on the Genus* Orchestoidea (*Crustacea*: *Amphipoda*) *in California*. Ph.D. Thesis, Stanford University, Palo Alto, California.

MCDONALD, J. H. 1984. *Ecological Genetics of Two Polymorphic Enzymes in Three Species of* Megalorchestia (*Amphipoda*: *Talitridae*). M.S. Thesis, Oregon State University, Corvallis, Oregon.

MITTON, J. B. AND GRANT, M. C. 1984. Associations among protein heterozygosity, growth rate and developmental homeostasis. *Annu. Rev. Ecol. Syst., 15*, 479–499.

NICHOLS, E. A. AND RUDDLE, F. H. 1973. A review of enzyme polymorphism, linkage and electrophoretic conditions for mouse and somatic cell hybrids in starch gels. *J. Histochem. Cytochem., 21*, 1066–1081.

NYMAN, L. 1975. Allelic selection in a fish (*Gymnocephalus cernua* (L.)) subjected to hotwater effluents. *Rep. Inst. Freshw. Res. Drottningholm, 54*, 75–82.

POULIK, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. *Nature, 180*, 1477–1479.

SASSAMAN, C. 1978. Dynamics of a lactate dehydrogenase polymorphism in the wood louse *Porcellio scaber* Latr.: evidence for partial assortative mating and heterosis in natural populations. *Genetics, 88*, 591–609.

SHAW, C. R. AND PRASAD, R. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochem. Gent., 4*, 297–320.

SINGH, S. M. AND GREEN, R. H. 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. *Malacologia, 25*, 569–581.

SMITH, P. J. AND FRANCIS, R. I. C. C. 1984. Glucosephosphate isomerase genotype frequencies, homozygous excess and size relationships in the sand flounder *Rhombosolea plebeia*. *Mar. Biol., 79*, 93–98.

SNEDECOR, G. W. AND COCHRAN, W. G. 1980. *Statistical methods*. Ames, Iowa: Iowa State Univ. Press. 507 pp.

SOKAL, R. R. AND ROHLF, F. J. 1981. *Biometry*. San Francisco, W. H. Freeman, 859 pp.

TRACEY, M. L., BELLET, N. F. AND GRAVEM, C. D. 1975. Excess allozyme homozygosity and breeding population structure in the mussel *Mytilus californianus*. *Mar. Biol., 32*, 303–311.

WATT, W. B., CASSIN, R. C. AND SWAN, M. S. 1983. Adaptation at specific loci. III. Field behavior and survivorship differences among *Colias* PGI genotypes are predictable from *in vitro* biochemistry. *Genetics, 103*, 725–739.

ZOUROS, E. AND D. W. FOLTZ. 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia, 25*, 583–591.

ZOUROS, E. AND KRIMBAS, C. B. 1969. The genetics of *Dacus oleae*. III. Amount of variation at two esterase loci in a Greek population. *Genet. Res., 14*, 249–258.

ZOUROS, E., SINGH, S. M. AND MILES, H. E. 1980. Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution, 34*, 856–867.