Interrelationships of Heterozygosity, Growth Rate and Heterozygote Deficiencies in the Coot Clam, *Mulinia lateralis*

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ABSTRACT

Allozyme surveys of marine invertebrates commonly report heterozygote deficiencies, a correlation between multiple locus heterozygosity and size, or both. Hypotheses advanced to account for these phenomena include inbreeding, null alleles, selection, spatial or temporal Wahlund effects, aneuploidy and molecular imprinting. Previous studies have been unable to clearly distinguish among these alternative hypotheses. This report analyzes a large data set (1906 individuals, 15 allozyme loci) from a single field collection of the coot clam *Mulinia lateralis* and demonstrates (1) significant heterozygote deficiencies at 13 of 15 loci, (2) a correlation between the magnitude of heterozygote deficiency at a locus and the effect of heterozygosity at that locus on shell length, and (3) a distribution of multilocus heterozygosity which deviates from that predicted by observed single-locus heterozygosities. A critical examination of the abovementioned hypotheses as sources of these findings rules out inbreeding, null alleles, aneuploidy, population mixing and imprinting as sole causes. The pooling of larval subpopulations subjected to varying degrees of selection, aneuploidy or imprinting could account for the patterns observed in this study.

NUMEROUS studies of natural populations of marine bivalves and other invertebrates have reported deficiencies of heterozygotes relative to Hardy-Weinberg expectations at allozyme loci [reviewed by ZOUROS and FOLTZ (1984a)]. In view of the population biology typical of many bivalve species (large effective population sizes, presumably random mating as a result of external fertilization, and widespread dispersal of pelagic larvae), the origin of such deficiencies is unclear. These findings are particularly puzzling when coupled with the observation, made in many of the same studies, that the degree of multiple-locus heterozygosity is positively correlated with fitness-related traits such as growth and viability [see MITTON and GRANT (1984) and ZOUROS and FOLTZ (1987) for reviews].

The possibility of a connection between these two phenomena was first suggested by ZOUROS, SINGH and MILES (1980), who noted that in the oyster *Crassostrea virginica*, only one allozyme locus (out of seven) showed no effect of heterozygosity on growth rate; this locus was also the only one which showed no heterozygote deficiency. We present here data from a large-scale study of the clam *Mulinia lateralis* which establish a clear relationship between the two phenomena, and allow us to discriminate among the numerous hypotheses that have been advanced to explain them.

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MATERIALS AND METHODS

Clams from a single field collection ($N = 3204$) of recently settled *Mulinia lateralis* were measured and scored electrophoretically at 15 polymorphic enzyme loci, as described by KOEHN, DIEHL and SCOTT (1988). From this sample complete size and genotype data were obtained for 1906 clams. All analyses refer to this data set unless otherwise indicated. In this report, initial size (shell length at time of collection, SL_i) is used as the sole measure of growth rate, as effects of genotype at individual loci on SL_i were more pronounced than genotypic effects on subsequent growth in the laboratory.

All loci had multiple alleles, ranging from three at SOD to 14 at GPI (Table 1). Extremely rare alleles ($p_i < 0.001$) were pooled with the allele of nearest electrophoretic mobility prior to statistical analyses. For each locus, observed genotypic distributions were compared with Hardy-Weinberg expectations (G-test for goodness of fit) (SOKAL and ROHLF 1981). Heterozygote deficiencies were expressed as $D = [H_o - H_e]/H_e$, where H_o is the observed frequency of heterozygotes and H_e the expected frequency. Heterogeneity of heterozygote deficiencies among loci was examined by treating them as correlation coefficients and testing accordingly (CROW and KIMURA 1970; SOKAL and ROHLF 1981).

For each locus, homogeneity of deficiencies of individual heterozygous genotypes was assessed by the goodness of fit of the observed genotypic distribution to that expected using a single inbreeding coefficient estimated from the observed genotypic distribution as $F = -D$. For graphical representation, allele-specific heterozygote deficiencies (D_j) were calculated by considering one allele at a time vs. all others pooled, using all available data for each locus. Because the resulting allelic D_j values are not independent (they are positively correlated), this presents a conservative illustra-

tion of heterogeneity of heterozygote deficiencies among alleles.

The sample population was subdivided into deciles on the basis of SL_i. For each decile, we calculated allele frequencies, allele frequencies within homozygotes, and heterozygote deficiencies for all major alleles ($p > 0.05$). These data are available from the senior author upon request. Heterogeneity of allele frequencies among size classes (deciles) was examined using $R \times C$ tests of independence (SOKAL and ROHLF 1981). Relationships between size (decile number) and heterozygote deficiencies were expressed as product-moment correlations. Genetic distances among deciles were estimated according to NEI (1978).

Within loci, genotypic effects on SL_i were examined by the regression of mean SL_i for each heterozygous genotype on the arithmetic mean of the mean SL_i of the two associated homozygotes. Because sample sizes and standard errors of mean SL_i varied greatly among genotypes, weighted least squares regression was employed. Each genotypic mean was weighted by the reciprocal of its standard error; for homozygotes, the arithmetic mean of the standard errors of the two homozygous genotypes was used. In order to obtain information on as many genotypes as possible, the original data set was used; only genotypes represented by five or more individuals were considered. To provide an adequate number of points for regression, only loci with more than three common (*i.e.*, homozygote frequency of five or greater) alleles were considered.

Individual loci were ranked according to the degree to which heterozygosity at each locus affected SL_i, using the partial sums of squares from the multiple regression of SL_i on individual locus heterozygosities (KOEHN, DIEHL and SCOTT 1988), in order to correlate heterozygote deficiencies at individual loci with the effects of heterozygosity on size. The magnitude of single-locus heterozygosity effects on SL_i was also quantified by comparing mean z-transformed SL_i of homozygotes and heterozygotes at each locus (Table 1).

Observed multiple-locus heterozygosity (MLH) distributions were determined by counting the number of individuals heterozygous for 0, 1, . . . k loci. Expected multiple-locus heterozygosity distributions were calculated on the basis of observed single-locus heterozygosities, assuming independence of heterozygosities among loci. Observed and expected distributions were compared by a G-test for goodness of fit (SOKAL and ROHLF 1981); some classes were pooled in order to obtain expected frequencies of five or greater for each heterozygosity class.

Dilocus heterozygosity associations were tested by a chi-square test of independence (SOKAL and ROHLF 1981). Expected heterozygosity distributions were calculated for all possible ($N = 105$) pairs of loci; deviations were expressed relative to the maximum deviation possible given the observed heterozygosities at the two loci in question, as $D'_H = D_H/D_H\text{max}$. D_H is defined as the excess of double heterozygotes (the observed frequency minus the product of the observed single-locus heterozygosities). $D_H\text{max}$ is equal to either the lesser of Hom_iHet_j or Het_iHom_j if D is positive, or to the lesser of Hom_iHom_j or Het_iHet_j if D is negative, where Hom_i is the observed homozygosity at the ith locus and the Het_j is the observed heterozygosity at the jth locus. This measure D'_H is analogous to the standardized gametic phase disequilibrium of LEWONTIN (1964) and may be termed the standardized dilocus heterozygosity disequilibrium.

A test of the hypothesis that the heterozygosity-size correlation is due to associative overdominance (the effects of heterozygosity at linked loci rather than the allozyme loci *per se*) was suggested by E. ZOUROS (personal communication). If associative overdominance is the source of heterozygote advantage, the effects of heterozygosity should be approximately additive between unlinked loci, and less than additive between linked loci (because linked loci share associated deleterious alleles). An individual locus heterozygosity effect was calculated as the difference in SL_i between homozygotes and heterozygotes. The expected dilocus heterozygosity effect was calculated as the sum of the two individual locus effects, for comparison with the observed dilocus heterozygosity effect, which was calculated as the difference in SL_i between double heterozygotes and double homozygotes. This test was applied to all pairwise combinations of loci which had significant individual effects on SL_i (except for IDH2, which was virtually monomorphic).

Effects of selection and mixing were examined by computer simulation of a population with five triallelic unlinked loci. Initial conditions were Hardy-Weinberg equilibrium at individual loci and linkage equilibrium. Viability selection could be imposed on either heterozygosity at individual loci or on MLH, after which the surviving population was examined for fit to Hardy-Weinberg equilibrium at individual loci and deviations of MLH from expectation based on observed single-locus heterozygosities.

In order to simulate a temporal Wahlund effect, a base population as described above was divided into two discrete spawning groups, on the basis of MLH. Within these groups, gametes united at random, after which progeny were pooled and examined as above.

The combined effects of population mixing and selection acting on individual loci were examined for two unlinked triallelic loci. Initially identical subpopulations were subjected to viability selection, after which they were pooled. Single-locus heterozygote deficiencies and dilocus heterozygosity disequilibrium were calculated from the total (pooled) population. Two models of viability selection were examined: underdominance and dominance of the less fit allele. In the former, viability of heterozygotes varied among subpopulations randomly from 0 to 1 and was the same at both loci in each subpopulation. In the latter, fitnesses of individual alleles varied randomly from 0 to 1, with the less fit allele always being completely recessive to the fitter allele; fitness arrays were identical at both loci.

RESULTS

Significant heterozygote deficiencies were observed at 13 of 15 loci (Table 1). The heterozygote deficiencies showed highly significant heterogeneity among loci ($\chi^2 = 394.1$, d.f. = 14, $P < 0.001$).

A significant correlation ($r = 0.703$, d.f. = 13, $P < 0.01$) was observed between the effects of heterozygosity at individual loci on SL_i and the magnitude of heterozygote deficiencies (Figure 1). Loci which showed the greatest heterozygote deficits were those at which heterozygosity had the greatest positive effect on SL_i.

Allele frequencies were not significantly heterogeneous among size deciles, with the exception of βGAL ($P \ll 0.001$). In contrast, the observed heterozygote deficiencies were size-dependent; smaller size classes showed larger deficiencies of heterozygotes (Table 2). Only loci which had large effects of heterozygosity on SL_i showed strong positive correlations between size and D (Table 2).

TABLE 1

Individual locus data

Locus	n_a	n_e	H	D	H-W	I	Z
ENOL	5	2.88	0.529	−0.190	$P < 0.001$	NS	0.384
AP1	9	4.00	0.570	−0.242	$P < 0.001$	NS	0.284
AP3	7	3.77	0.438	−0.405	$P < 0.001$	$P < 0.001$	0.270
PGM	9	2.26	0.484	−0.131	$P < 0.001$	$P < 0.001$	0.236
IDH2	4	1.01	0.008	−0.115	$P < 0.001$	NA	0.989
AP2	11	2.86	0.532	−0.182	$P < 0.001$	$P < 0.005$	0.196
MPI	11	4.67	0.613	−0.220	$P < 0.001$	$P < 0.001$	0.150
βGAL	10	5.21	0.586	−0.275	$P < 0.001$	$P < 0.001$	0.139
SOD	3	1.09	0.079	−0.042	NS	NA	0.166
GPI	14	3.08	0.654	−0.053	$P < 0.001$	$P < 0.05$	0.081
AAP	11	3.92	0.648	−0.130	$P < 0.001$	$P < 0.05$	0.091
MDH2	5	1.09	0.065	−0.153	$P < 0.001$	NS	0.061
MDH1	4	1.04	0.035	−0.080	$P < 0.001$	NS	0.031
IDH1	7	2.05	0.517	0.008	NS	NS	−0.012
PGD	6	2.02	0.458	−0.094	$P < 0.001$	NS	−0.020

n_a = absolute number of alleles observed per locus; n_e = effective number of alleles; H = observed heterozygosity; D = heterozygote deficiency; H-W = G-test of goodness of fit to Hardy-Weinberg equilibrium genotype frequencies; I = G-test of goodness of fit of observed genotype frequencies to frequencies expected using an inbreeding coefficient of $F = -D$; Z = mean z-transformed SL_i of heterozygotes minus mean z-transformed SL_i of homozygotes. Loci ordered according to effect of heterozygosity on SL_i (see MATERIALS AND METHODS).

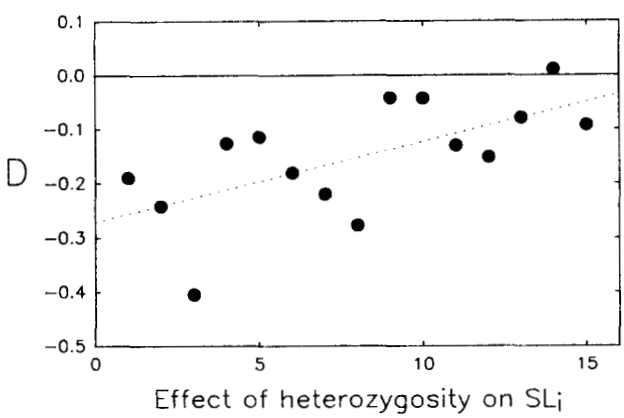

FIGURE 1.—Heterozygote deficiencies in relation to the effect of heterozygosity on SL_i. Average locus effect (rank) and D are significantly correlated ($r = -0.703$, d.f. = 13, $P < 0.01$).

Within loci, allele-specific heterozygote deficiencies (D_j) were significantly heterogeneous in five cases: AP3 ($P \ll 0.001$), PGM ($P \ll 0.001$), AP2 ($P < 0.005$), MPI ($P < 0.001$) and βGAL ($P < 0.001$). In addition, GPI and AAP showed slightly significant heterogeneity ($P < 0.05$). Within loci, allelic heterozygote deficiencies (D_j) were negatively correlated with allele frequency at three loci: ENOL (5 alleles, $r = -0.911$, $P < 0.05$), AAP (7 alleles, $r = -0.863$, $P < 0.01$) and MPI (8 alleles, $r = -0.906$, $P < 0.01$). Common alleles showed the largest deficiencies.

Analysis of individual alleles showed a positive relationship between heterozygote deficiency and size class at 34 of 39 alleles examined; 17 of these showed significant correlations at the 0.05 level or greater, while none of the negative correlations was significant. Overall, alleles which showed large heterozygote deficiencies showed stronger correlations between D_j and size class ($r = -0.51$, d.f. = 37, $P < 0.001$).

Within loci, there was generally a positive correlation between the mean SL_i of a particular heterozygote and the mean SL_i of the two associated homozygotes. Weighted regression analysis showed positive slopes for MPI ($P < 0.001$), AAP ($P < 0.001$), AP1 ($P < 0.001$), βGAL ($P < 0.001$), AP3 ($P = 0.007$), and AP2 ($P = 0.049$). Only GPI and PGM showed nonsignificant regressions. Of the six significant slopes, all but one (AAP) had values significantly less than one, indicating that the heterotic effect on SL_i was greater for alleles associated with lower mean homozygote values of SL_i.

The observed MLH distribution deviated significantly ($G = 31.3$, d.f. = 9, $P < 0.001$) from the expected distribution, showing excesses of highly homozygous and highly heterozygous individuals and a deficiency of individuals of intermediate heterozygosity (Figure 2). The 15 loci examined can be subdivided into a group of eight loci which had significant individual effects on SL_i, and a group of seven which did not (KOEHN, DIEHL and SCOTT 1988). The former group shows the characteristic MLH disequilibrium ($G = 21.3$, d.f. = 7, $P < 0.005$; Figure 3), while the latter group does not (Figure 4).

Another way of describing interlocus associations is by comparing the variance of k, the number of heterozygous loci per individual, with the variance expected in a panmictic population with a given mean heterozygosity (BROWN, FELDMAN and NEVO 1980). In this collection of *M. lateralis*, the mean number of loci heterozygous per individual was 6.215, with a variance of 3.335. The latter significantly exceeds the

TABLE 2

Heterozygote deficiencies in size deciles (ordered from smallest to largest) of *M. lateralis*

Locus	Decile										
	1	2	3	4	5	6	7	8	9	10	*r*
ENOL	−0.42	−0.39	−0.29	−0.24	−0.22	−0.11	−0.07	−0.17	−0.03	−0.05	0.954***
AP1	−0.34	−0.39	−0.28	−0.23	−0.22	−0.24	−0.24	−0.25	−0.14	−0.08	0.872***
AP3	−0.55	−0.59	−0.44	−0.38	−0.46	−0.36	−0.41	−0.31	−0.29	−0.25	0.911***
PGM	−0.20	−0.20	−0.32	−0.10	−0.09	−0.08	−0.11	−0.09	−0.06	−0.01	0.791*
IDH2	*M*	0.00	0.00	0.00	−1	*M*	0.00	0.00	0.00	0.01	0.144
AP2	−0.35	−0.25	−0.18	−0.21	−0.21	−0.12	−0.15	−0.14	−0.18	−0.02	0.836**
MPI	−0.33	−0.30	−0.25	−0.20	−0.19	−0.19	−0.16	−0.16	−0.21	−0.18	0.813**
βGAL	−0.36	−0.30	−0.27	−0.32	−0.25	−0.30	−0.21	−0.26	−0.31	−0.16	0.653*
SOD	0.04	−0.16	0.03	0.03	0.04	−0.20	0.05	−0.05	−0.01	−0.16	−0.243
GPI	−0.09	−0.02	−0.08	−0.01	−0.08	−0.04	0.00	−0.04	−0.08	0.02	0.420
AAP	−0.12	−0.12	−0.24	−0.18	−0.14	−0.08	−0.07	−0.19	−0.05	−0.07	0.462
MDH2	0.02	−0.08	−0.21	0.02	−0.13	−0.42	−0.09	−0.29	−0.10	0.03	−0.133
MDH1	0.02	0.01	−0.18	−0.28	0.01	0.02	−0.07	−0.32	0.02	0.01	−0.005
IDH1	−0.02	0.07	0.04	0.02	0.01	0.02	−0.06	0.02	0.01	0.01	−0.254
PGD	−0.17	−0.13	0.01	−0.17	−0.06	−0.07	−0.07	−0.04	−0.14	−0.08	0.258

Loci ordered by contribution of individual locus heterozygosity to SL_i. *r* = product-moment correlation between decile number and *D*. *M* = monomorphic.
*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

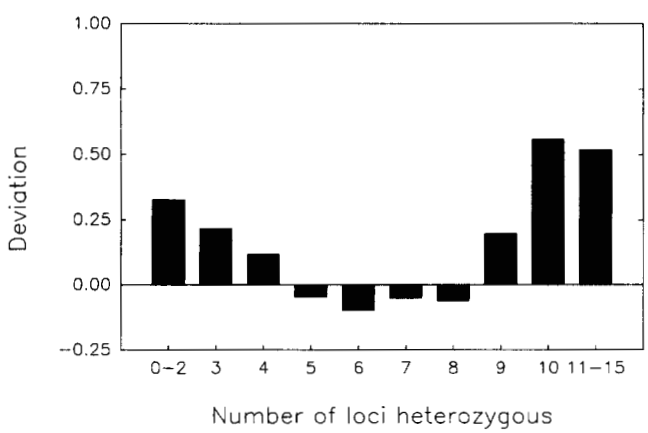

FIGURE 2.—Significant deviation ($P < 0.001$) of observed multilocus heterozygosity distribution from expected distribution based on observed single-locus heterozygosities at all 15 loci.

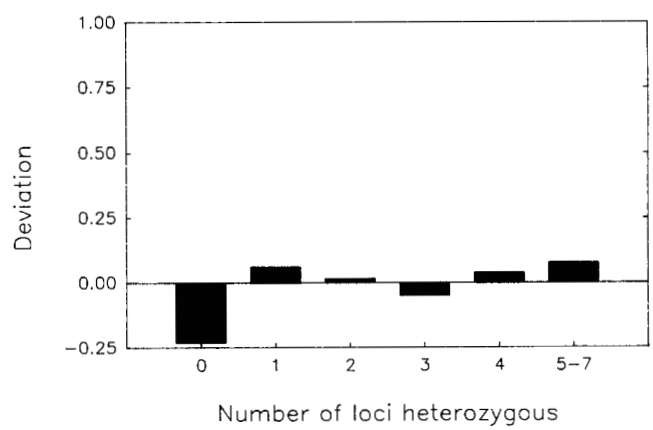

FIGURE 3.—Significant deviation ($P < 0.005$) of observed multilocus heterozygosity distribution from expected distribution based on observed single-locus heterozygosities at eight loci which have significant effects on SL_i.

variance expected from the observed single-locus heterozygosities (expected variance = 2.846, with a 95% upper confidence limit of 3.025).

This "heterozygosity disequilibrium" is only weakly evident at the level of dilocus combinations. Of the eleven (out of 105) possible combinations which showed disequilibria significant at the 0.05 level, all were characterized by excesses of double homozygotes and double heterozygotes (Table 3); however, no dilocus heterozygosity association was significant at the Bonferroni adjusted probability level of 0.0005 (=0.05/105). The mean dilocus heterozygosity disequilibrium (D_H) value was significantly different from zero (mean ± 95% C.I. = 0.0023 ± 0.0009), while the mean standardized disequilibrium (D'_H) was not significantly different from zero (0.0280 ± 0.0288).

The mean SL_i of double heterozygotes was on average only slightly less than predicted (96.9% ± 1.4% 95% C.I.) by summing the effects of heterozygosity at individual loci; observed and predicted values were generally in good agreement (21 pairs of loci, $r = 0.993$, $P < 0.001$). Deviations for pairs of linked loci (ENOL-PGD and AP2-IDH1; T. M. SCOTT, unpublished results) were not different from other pairs of presumably unlinked loci.

Distinguishing null homozygotes from experimental artifacts was not feasible in a large-scale survey such as this. Null heterozygotes are electrophoretically indistinguishable from wild-type homozygotes in the case of monomeric enzymes; in the case of CRM^+ dimeric enzymes, however, null heterozygotes can be detected by the appearance of a 2-banded heterozygote instead of the expected 3-band pattern. At the GPI locus, five individuals (of 1906) were apparently null heterozygotes (estimated null allele frequency *x*

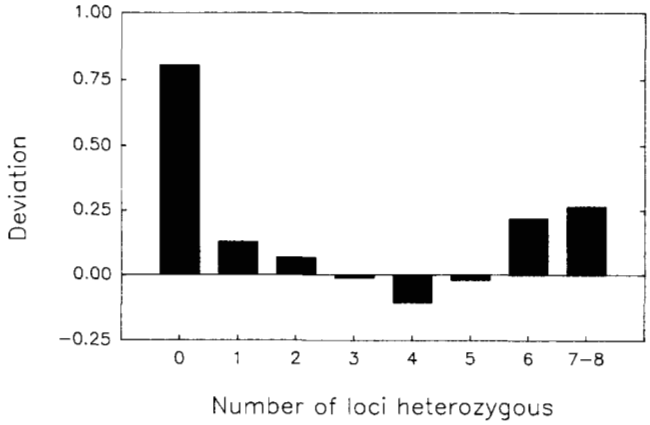

FIGURE 4.—Deviation of observed multilocus heterozygosity distribution from expected distribution based on observed single-locus heterozygosities at seven loci which do not have significant effects on SL_4. Observed and expected MLH distributions are not significantly different.

= 0.001). At the AP2 locus only one null heterozygote was identified (x = 0.0003). No null heterozygotes were observed at the other dimeric loci.

DISCUSSION

Three striking patterns were observed: (1) heterogeneous but generally large heterozygote deficiencies at nearly all loci, (2) multilocus heterozygosity disequilibrium, characterized by excesses of highly homozygous and highly heterozygous individuals, and (3) a correlation between the degree of heterozygote deficiency and the effect of heterozygosity on size.

Sources of heterozygote deficiencies and heterozygosity disequilibrium

Heterozygote deficiencies have been noted in almost 50 bivalve species (FUJIO, YAMANAKA and SMITH 1983; ZOUROS and FOLTZ 1984a). In *M. lateralis*, significant heterozygote deficiencies are found at almost all loci. Smaller individuals tend to have larger heterozygote deficiencies; a similar pattern has been reported for several other bivalves including *Mytilus californianus* (TRACEY, BELLET and GRAVEM 1975), *Crassostrea virginica* (SINGH and ZOUROS 1978; ZOUROS, SINGH and MILES 1980), *Mytilus edulis* (KOEHN and GAFFNEY 1984) and *Geukensia demissa* (KOEHN, TURANO and MITTON 1973). This pattern is generally attributed to the higher growth rate and viability of heterozygotes.

The correlation between the magnitude of heterozygote deficiency and the effect of heterozygosity on size is a novel finding. Any hypothesis which attempts to explain the origin of heterozygote deficiencies or the heterozygosity-size correlation must also account for this observation.

The multilocus "heterozygosity disequilibrium" observed in *M. lateralis* has not been reported in other allozyme surveys of animals, although most studies

TABLE 3

Absolute and standardized dilocus heterozygosity disequilibrium values for 11 pairs of loci which show significant heterozygosity associations

Loci	D_H	D'_H	P
ENOL-PGD	0.014	0.063	0.017
ENOL-MDH2	0.006	0.196	0.034
ENOL-AP1	0.012	0.052	0.036
ENOL-AP2	0.014	0.057	0.014
ENOL-SOD	0.007	0.179	0.031
AP1-MPI	0.011	0.050	0.047
AP3-GPI	0.013	0.086	0.016
AP3-AP2	0.014	0.066	0.017
IDH2-AAP	0.002	0.811	0.020
MPI-AAP	0.011	0.050	0.043
βGAL-IDH1	0.017	0.079	0.003

P = probability from chi-square test of independence.

lack sufficiently large sample sizes to yield an adequate test. In the oyster *C. virginica*, the observed MLH distribution at seven allozyme loci was in accord with that predicted on the of basis single-locus heterozygosities (ZOUROS, SINGH and MILES 1980; FOLTZ, NEWKIRK and ZOUROS 1983). Calculations of MLH disequilibria from published data (single-locus heterozygosities and observed MLH distributions) show a significant MLH disequilibrium in the gastropod *Thais haemastoma* (GARTON 1984) similar to that shown in Figure 2. By contrast, several teleost species show negligible heterozygote deficiencies and no MLH disequilibria (Table 4).

In virtually all studies that have attempted to explain the origin of heterozygote deficiencies or the heterozygosity-growth correlation in natural populations of marine bivalves, the data obtained were inadequate to distinguish among various alternative hypotheses. Because of the large sample sizes (number of individuals as well as number of polymorphic loci) employed in this data set, we are able to critically evaluate these hypotheses as sources of the patterns observed here.

Misscoring: Poor electrophoretic resolution may result in the misscoring of heterozygotes as homozygotes, yielding an apparent heterozygote deficiency (AYALA *et al.* 1973). If smaller individuals are more readily misscored, owing to an inadequate mass of tissue, a correlation between size and heterozygote deficiency might appear. Enzyme loci which stain poorly would show greater apparent heterozygote deficiencies and a greater correlation between size and heterozygote deficiency than well-resolved loci.

One test of the hypothesis that individuals difficult to score are more likely to be recorded as homozygotes is to compare frequencies of homozygotes in the original data set (N = 3204) and the data set used (N = 1906). If individuals which were not scored at all 15 loci were more likely to be misscored than those

TABLE 4

Allozyme heterozygote deficiencies and MLH deviations in other marine species

Species	N	N_L	N_D	D	MLH deviation	Source
Thais haemastoma (oyster drill)	286	6	5	−0.41	$P < 0.025$	GARTON (1984)
Crassostrea virginica (oyster)	1699[a]	7	6	−0.30	NS	ZOUROS, SINGH and MILES (1980)
Clupea harengus (Atlantic herring)	1405	13	1	−0.004	NS	RYMAN *et al.* (1984)
Pleuronectes platessa (plaice)	4199	5	0	−0.008	NS	WARD and BEARDMORE (1977); MCANDREW, WARD and BEARDMORE (1982)
Oncorhynchus keta (chum salmon)	2809	7	NA[b]	14/216[b]	NS	BEACHAM and WITHLER (1985) and GOULD (1985)
Fundulus heteroclitus (killifish)	997	12	4	−0.21[c]	NS	MITTON and KOEHN (1975)

N = sample size, N_L = number of loci examined, N_D = number of loci showing significant heterozygote deficiencies, D = mean heterozygote deficiency across loci.

[a] Mean sample size across loci.

[b] D values not published; 14 of 216 population analyses showed nominally ($P < 0.05$) significant heterozygote deficiencies, distributed across 6 of 7 loci.

[c] Data provided for one locus (EST-3) only, calculated from Table 5 of MITTON and KOEHN (1975).

which were scored at all loci, we would expect the former category to show higher frequencies of homozygotes. In fact there were no significant differences in frequencies at any locus except SOD, which contrary to expectation showed a lower frequency of homozygotes in the original data set.

In addition, there appears to be no relation between the degree of staining resolution of individual allozyme loci and their observed heterozygote deficiencies. In the original data set, the proportion of individuals which were not scored ranged among loci from 1.8% at GPI to 15.6% at AP1. Overall, the relationship between heterozygote deficiency and proportion not scored was not significant ($r = -0.31$, d.f. = 13, NS). We conclude that scoring bias is of negligible importance in this study.

Inbreeding: In species with separate sexes, partial inbreeding may occur through the mating of related individuals (HEDRICK and COCKERHAM 1986). The resulting increase in homozygosity for rare, detrimental alleles in inbred individuals would lead to a positive association between individual heterozygosity and fitness (STRAUSS 1986). In addition, inbreeding may generate heterozygosity disequilibria among loci, with excesses of double homozygotes and double heterozygotes (HALDANE 1949). However, for organisms which have external fertilization and extended dispersal of planktonic larvae (most marine invertebrates), mating of relatives is generally considered to occur with negligible frequency.

Inbreeding affects all loci equally, generating uniform heterozygote deficiencies regardless of gene frequencies; within loci, all alleles are expected to show similar heterozygote deficiencies. Thus inbreeding as a source of heterozygote deficiencies may be directly tested by comparing heterozygote deficiencies both among loci and among alleles within loci. In our data, deficiencies were significantly heterogeneous among loci, and in five cases, among alleles within loci (Table 1; Figure 5). Indeed, some individual heterozygous genotypes were in excess. Thus although a minor contribution of inbreeding cannot be excluded, it cannot be a major factor in these data.

Null alleles: An individual heterozygous for a null allele will be misscored as a homozygote (MILKMAN and BEATY 1970). The relationship between null allele frequency (x) and heterozygote deficiency (D) is given by $D = -2x/[1 + x]$, assuming the population is in Hardy-Weinberg equilibrium and null homozygotes are absent, either because they are lethal or because they have been excluded from the data (SKIBINSKI, BEARDMORE and CROSS 1983). If null alleles are deleterious, the average fitness of individuals scored as homozygotes will be lower than that of heterozygotes, creating a positive correlation between degree of heterozygosity and fitness. This hypothesis has generally been rejected because it requires either extremely high mutation rates, or strong selection in favor of null heterozygotes in order to maintain null alleles at even moderate frequencies (ZOUROS, SINGH and MILES 1980; ZOUROS, ROMERO-DOREY and MALLET 1988).

The null allele frequencies estimated here for the GPI and AP2 loci are comparable to frequencies inferred to exist in natural populations of *Mytilus edulis* (SKIBINSKI, BEARDMORE and CROSS 1983) and measured directly in *Drosophila* (VOELKER *et al.* 1980; LANGLEY *et al.* 1981) and pine trees (ALLENDORF, KNUDSEN and BLAKE 1982). These frequencies are orders of magnitude too small to generate the large heterozygote deficiencies observed in this study.

The unusually high frequencies of null alleles postulated by FOLTZ (1986) to exist in the American oyster could also be attributed to a high level of

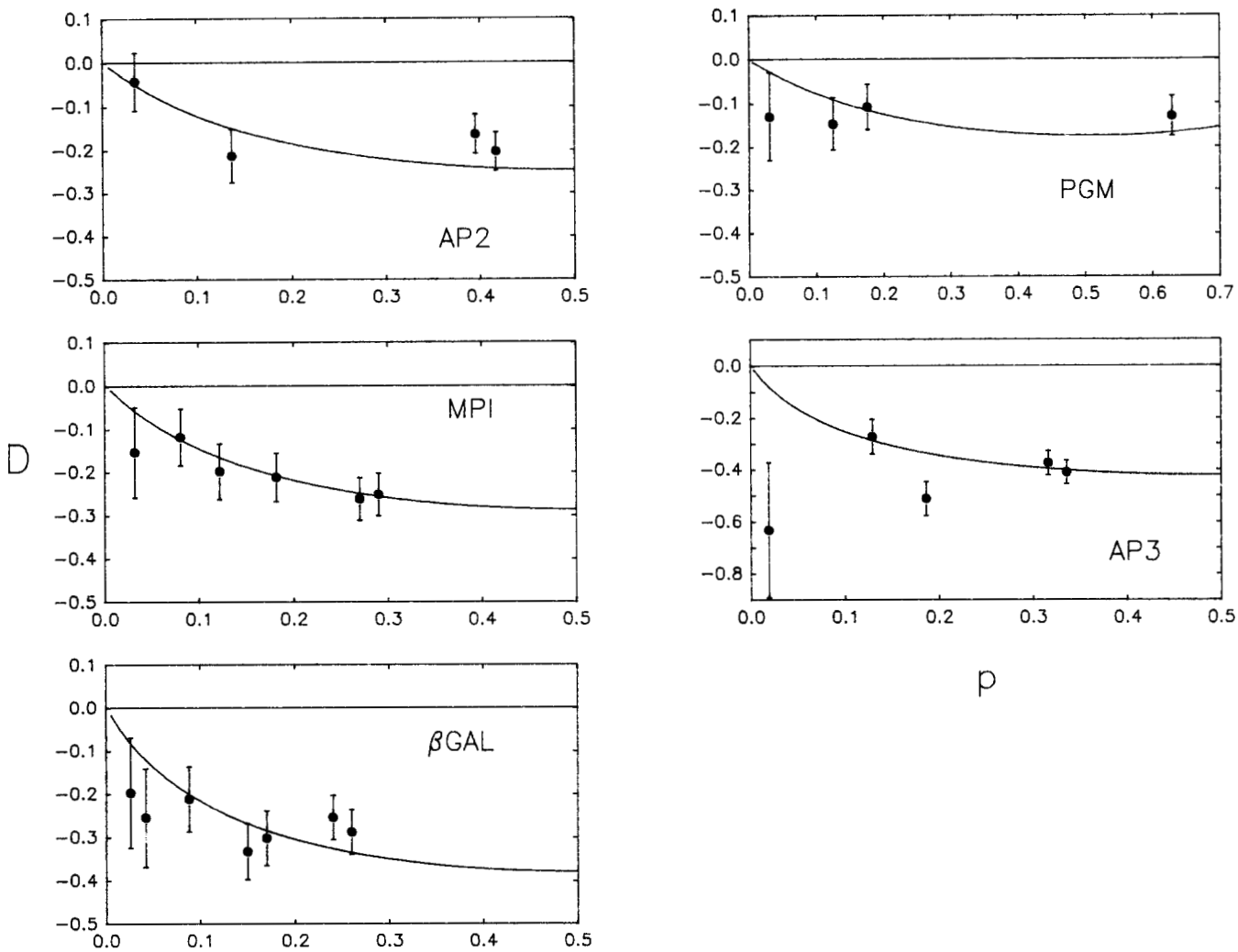

FIGURE 5.—Heterozygote deficiencies (±95% C.I.) calculated for individual alleles in relation to allele frequency. Only loci which showed significant heterogeneity of heterozygote deficiencies are shown. Curves depict relationship expected under models of selection against heterozygotes or against dominant alleles. (W_H = viability of heterozygotes relative to homozygotes; W_D = viability of dominant allele relative to recessive homozygote.) For AP2, $W_H = 0.60$, $W_D = 0.35$; for MPI, $W_H = 0.55$, $W_D = 0.30$; for βGAL, $W_H = 0.45$, $W_D = 0.20$; for PGM, $W_H = 0.70$, $W_D = 0.50$; for AP3, $W_H = 0.55$, $W_D = 0.30$.

spontaneous aneuploidy. From genotype distributions in progeny, he inferred that in some cases parents originally scored as homozygotes must have been null heterozygotes, as they appeared to have contributed no allele to some of their progeny. Alternative explanations are (1) the parent was monosomic for the chromosome or segment carrying the scored locus, or (2) the parent spontaneously produced aneuploid gametes. Electrophoretic evidence for the latter occurrence has been found in *Mytilus edulis*, where in one pair mating a male heterozygous at an aminopeptidase locus contributed neither allele to approximately one-third of its offspring (P. M. GAFFNEY, unpublished results).

Aneuploidy: ZOUROS and FOLTZ (1984b) suggested that the generation of aneuploid gametes by chromosome loss during meiosis could account for both heterozygote deficiencies (monosomics will be hemizygous for loci contained on the missing chromosome) as well as lower mean fitness of apparent homozygotes. Formation of aneuploid gametes in bivalves may be common, particularly under environmental stress (DIXON 1982). THIRIOT-QUIÉVREUX (1986) reported a high frequency of aneuploidy resulting from chromosome loss in juveniles of two oyster species, and suggested that the occurrence of aneuploidy could generate the heterozygote deficiencies commonly observed in bivalves. She also noted that slow-growing oyster spat had higher frequencies of aneuploid mitotic spreads than fast-growing spat, suggesting a relationship between apparent homozygosity and growth rate. However, this mechanism cannot explain heterozygote deficiencies. The differences in karyotypes reported by THIRIOT-QUIÉVREUX were in terms of relative frequencies of normal vs. aneuploid mitotic spreads *within* an individual. Unless the great

majority of cells of an individual were missing the chromosome carrying the scored locus, a heterozygote would still be correctly scored.

The frequency of aneuploidy observed in natural populations of bivalves (DIXON 1982) is sufficient to generate large heterozygote deficiencies. In laboratory-spawned *M. lateralis*, aneuploid eggs and early embryos, the majority of which have missing rather than extra chromosomes, occur with high frequency (RUPRIGHT 1983; SCARPA 1985). Heterozygote deficiencies will vary among loci if certain chromosomes or chromosome segments are more likely to be lost than others; those loci most affected are those at which the apparent superiority of heterozygotes would be greatest. This would explain the correlation between locus-specific heterozygote deficiencies and effects on size.

If some subpopulations sustained high frequencies of aneuploidy owing to local environmental stresses while other subpopulations experienced little or no aneuploidy, their mixing at the time of larval settlement would produce a positive covariance of heterozygosities among loci (including unlinked loci), resulting in a MLH disequilibrium of the type seen in *M. lateralis*. Pairs of loci in which both were prone to aneuploidy would show larger dilocus heterozygosity disequilibria than less affected pairs; this would generate a correlation between the mean single-locus heterozygote deficiencies of pairs of loci and their dilocus disequilibria. For the 105 dilocus combinations, a significant correlation ($r = -0.30$, $P < 0.002$) between the mean single-locus D and the dilocus disequilibrium (D_H) was observed.

The aneuploidy hypothesis may be further tested with the available data. First, loss of a chromosome or segment thereof would create apparent heterozygote deficiencies of comparable magnitude at all loci present on the chromosome or segment, independent of allele frequencies. From preliminary data, two linkage groups are known among the 15 loci included in this study: ENOL-PGD and AP2-IDH1 have estimated recombination frequencies of 5.5% and 20%, respectively, (T. M. SCOTT, unpublished results). In each of these pairs one of the loci exhibited a large heterozygote deficiency (ENOL, AP2) while the other member of the pair did not deviate significantly from Hardy-Weinberg proportions. These observations are compatible with the aneuploidy hypothesis only if a typical aneuploid segment is so small that it does not include both loci.

A second test is provided by examining heterozygote deficiencies with respect to individual alleles. Like inbreeding, aneuploidy generates heterozygote deficiencies that are invariant with regard to allele frequency. In contrast, allelic D values are heterogeneous at several loci (Table 1; Figure 5).

Finally, if aneuploid individuals were more often represented among the homozygotes at a given locus, the shapes of the size-frequency distributions of homozygotes and heterozygotes might be expected to differ in a regular way, *e.g.*, the distribution of homozygotes being more skewed and platykurtic. There were no systematic differences in SL_i between homozygotes and heterozygotes with respect to either g_1, g_2 or coefficients of variation. We conclude that aneuploidy cannot be the sole source of the patterns observed here.

Molecular imprinting: CHAKRABORTY (1989) noted that the phenomenon of molecular imprinting (differential expression of a gene based on whether it is maternally or paternally derived) may result in both heterozygote deficiencies and apparent heterosis, with population genetic consequences similar to those of null alleles, aneuploidy and incomplete penetrance. The considerations discussed above under Aneuploidy also apply to molecular imprinting.

As with aneuploidy, one would expect molecular imprinting to produce heterozygote deficiencies which are identical among alleles at a single locus, in contrast to the heterogeneity observed (Figure 5). In addition, for imprinting alone to generate the covariance of heterozygosities necessary for dilocus heterozygosity disequilibrium, it would require either the mixing of populations with different proportions of imprinting, or the restriction of imprinting to a fraction of the population. Thus imprinting is also insufficient as a sole explanation of the patterns observed in *M. lateralis*.

Selection: Selection against heterozygotes during larval life followed by selection in favor of heterozygotes after settlement has been postulated to account for the appearance of heterozygote deficiencies in juveniles and the subsequent emergence of a heterozygote advantage (ZOUROS *et al.* 1983; MALLET *et al.* 1985). MALLET *et al.* (1985) observed slight heterozygote deficiencies at two allozyme loci in progeny of pair matings of *Mytilus edulis*, and postulated that genotype-dependent larval mortality was the most likely cause. Two modes of selection could account for this result: (1) underdominance for viability (*i.e.*, selection against heterozygotes), or (2) dominance of the unfavorable allele (ZOUROS and FOLTZ 1984b).

Either of these modes of selection may account for the slight deficiencies observed in MALLET *et al.* (1985). However, in order to obtain the large deficiencies observed in *M. lateralis*, extreme genotypic differences in larval viability would be required. For the loci examined in this study, the first model would require heterozygote viabilities in the range of 0.5–0.9 to produce the observed deficiencies, while the second model would require fitnesses of the dominant allele in the range of 0.25–0.75 (Figure 5). Fitness

TABLE 5

Effect of selection and temporal spawning separation on MLH distribution and single-locus heterozygote deficiencies

A. Effects of selection on multilocus heterozygosity on the deviation of observed MLH distribution from that predicted by single-locus heterozygosities. D_{min} and D_{max} = smallest and largest values of single-locus heterozygote deficiencies observed among the five loci.

	Number of loci heterozygous							
Effect	0	1	2	3	4	5	D_{min}	D_{max}
Viability	0.5	0.6	0.7	0.8	0.9	1		
Deviation	−0.06	−0.01	0.01	0.01	−0.01	−0.03	0.03	0.07
Viability	1	0.9	0.8	0.7	0.6	0.5		
Deviation	−0.04	−0.01	0.01	0.01	−0.01	−0.06	−0.07	−0.03
Viability	0.5	0.75	1	1	0.75	0.5		
Deviation	−0.45	−0.18	0.11	0.12	−0.15	−0.42	−0.01	0.01
Viability	1	0.75	0.5	0.5	0.75	1		
Deviation	0.70	0.27	−0.16	−0.17	0.21	0.55	−0.01	0.02

B. Effect of dividing population into two temporally separated spawning groups on the basis of multilocus heterozygosity. Effort = proportion of total gametes shed in spawning period 1, with remainder shed in spawning period 2.

	Number of loci heterozygous							
Effect	0	1	2	3	4	5	D_{min}	D_{max}
Effort	0	0	0	1	1	1		
Deviation	0.06	0.02	−0.01	−0.02	0.01	0.10	−0.03	0
Effort	0	0.2	0.4	0.6	0.8	1		
Deviation	0.01	0.01	0	0	0	0.02	−0.01	0

Initial allele frequencies: $p_1 = 0.05$, $q_1 = 0.15$, $r_1 = 0.80$; $p_2 = 0.10$, $q_2 = 0.20$, $r_2 = 0.70$; $p_3 = 0.20$, $q_3 = 0.30$, $r_3 = 0.50$; $p_4 = 0.30$, $q_4 = 0.30$, $r_4 = 0.40$; $p_5 = 0.50$, $q_5 = 0.50$, $r_5 = 0.00$.

differences associated with allozyme loci are typically several orders of magnitude smaller than these (EANES 1987), and are often attributed to linkage disequilibrium with deleterious recessive alleles. This mechanism cannot be invoked to explain underdominance, however; instead, linkage disequilibrium with a truly underdominant locus must be postulated.

Heterozygosity disequilibrium does not result from selection acting independently on individual loci, but may occur when viability varies among individuals according to their MLH. In computer simulations, a variety of results is obtained when viability is a monotonic function of MLH. Very stringent selection against heterozygosity produces only modest single-locus heterozygote deficiencies as well as deficiencies in the tails of the MLH distribution, the opposite of the pattern observed (Figure 2). Selection in favor of MLH results in heterozygote excesses at individual loci, and again, deficiencies in the tails of the MLH distribution (Table 5A).

Selection in favor of intermediate levels of heterozygosity (against low and high heterozygosity) produces marked deficits in the tails of the MLH distribution, and negligible deviations from Hardy-Weinberg equilibrium at individual loci. Selection against intermediate levels of heterozygosity produces excesses in the tails of the MLH distribution (as we have observed), but only negligible excesses or deficiencies at individual loci. None of these results resembles our data; therefore, selection acting on individual MLH cannot account for the patterns we have observed.

Wahlund effect: The pooling of subpopulations which differ in allele frequencies produces a deficiency of heterozygotes in the combined population, in proportion to the variance of allele frequencies among the subpopulations (WAHLUND 1928). For multiallelic loci, allele-specific heterozygote deficiencies may thus vary in magnitude; in addition, some heterozygous genotypes may actually exceed expected frequencies. Both heterogeneity in allele-specific deficiencies and excesses of particular heterozygous genotypes were observed. However, deficiencies of the magnitude observed in this study would require extraordinarily large variances in allele frequencies. For example, in the two-allele case where p varies randomly from 0 to 1 among subpopulations (*i.e.*, a uniform distribution), D in the total population equals −0.33, a value comparable to those observed in *M. lateralis* and other bivalve species. Yet for most allozyme loci in bivalves, large-scale geographic variation in allele frequencies is relatively modest. This militates against population mixing as a major source of large heterozygote deficiencies (ZOUROS and FOLTZ 1984a; JOHNSON and BLACK 1984), although in some cases spatial variation in allele frequency may be sufficient to produce mild deficiencies (KOEHN, MILKMAN and MITTON 1976). In any case, conventional geographic survey data on spatial variation in allele frequencies

are inadequate for evaluating this hypothesis. The critical data—allele frequencies in the larval subpopulations which combine at settlement—have not yet been obtained.

MLH disequilibrium may also be produced by population mixing. For two loci, the total disequilibrium is equal to $D_H = \bar{D}_H + \text{cov}(H_1,H_2)$, where $\bar{D}_H$ is the average dilocus heterozygosity disequilibrium value over k subpopulations, and $\text{cov}(H_1,H_2)$ is the covariance between heterozygosities at loci 1 and 2. Thus dilocus heterozygosity disequilibrium may be generated by mixing subpopulations in equilibrium if heterozygosities at the two loci covary, or if the subpopulations themselves are in heterozygosity disequilibrium. This principle extends to multiple loci. For example, mixing of two populations in which the heterozygosity at each locus in the first population is 0.1 higher than that of the second population will produce a pattern of MLH distribution comparable to the one observed here.

TRACEY, BELLET and GRAVEM (1975) proposed that heterozygote deficits in marine invertebrates could result from the temporary random subdivision of a population into small reproductive groups, within which panmixia obtained. Allele frequency differences among reproductive groups would result simply from sampling error, leading to a Wahlund effect when progeny were pooled. However, as PUDOVKIN and ZHIVOTOVSKII (1980) pointed out, binomial sampling in small breeding groups produces differences in allele frequencies between males and females, which acts to counterbalance the heterozygote deficiency generated by mixing of randomly differentiated reproductive groups. Thus the transient formation of small breeding groups within a homogenous population cannot generate heterozygote deficiencies.

A Wahlund effect may be generated by the temporal subdivision of a population into discrete spawning groups, if the groups differ in allele frequencies. In the simplest case, a single locus with two alleles, the formation of two breeding groups (heterozygotes and homozygotes) can generate large heterozygote deficiencies when allele frequencies are skewed (ZOUROS and FOLTZ 1984b). HILBISH and ZIMMERMAN (1988) have demonstrated differences in the timing of spawning among genotypes at the LAP locus in a population of *Mytilus edulis* capable of generating large heterozygote deficiencies. However, they suggest that this phenomenon is probably not the cause of heterozygote deficiencies often encountered at this locus in *M. edulis*, as it occurs only under restricted conditions, in a steep gene frequency cline maintained by selection at the LAP locus.

It proves to be difficult to generalize the temporal Wahlund effect from the single-locus two-allele model to multiple alleles or to multiple loci. In the case of multiple alleles at one locus, only modest heterozygote deficiencies result from the complete spawning separation of homozygotes and heterozygotes, particularly if the alleles are evenly distributed. When the model is extended to multiple loci, homozygotes cannot be separated from heterozygotes at all loci simultaneously without creating numerous breeding groups. If a population is subdivided into two temporally separated breeding groups on the basis of heterozygosity (*i.e.*, spawning time is a function of individual MLH), slight single-locus heterozygote deficits as well as excesses in the tails of the MLH distribution are produced (Table 5B), but they are an order of magnitude smaller than those observed here in *M. lateralis*. A similar pattern emerges if a single population spawns twice, with one of the spawnings being limited to a subset of the total population (*e.g.*, highly heterozygous individuals). We therefore conclude that temporal subdivision of a single panmictic population on the basis of MLH is incapable of generating either large single-locus heterozygote deficiencies at several loci simultaneously, or significant departures from the expected MLH distribution.

In brief, the hypotheses of misscoring, inbreeding, null alleles, aneuploidy and molecular imprinting are inadequate as sole explanations of the various genetic patterns we have reported. Intense selection against heterozygotes would to produce the large heterozygote deficiencies observed; however, selection alone cannot simultaneously cause both single-locus heterozygote deficiencies and the MLH disequilibrium. While the Wahlund effect can produce both, it cannot account for the magnitude of the deviations observed, nor the relationship between MLH and size. We must consider a combination of these mechanisms to explain the patterns we have observed. In particular, combinations of subpopulation mixing and other factors merit special attention.

Pooling of differentiated subpopulations is a potential contributor to single-locus heterozygote deficiencies and could also produce the MLH disequilibria observed, if heterozygosities among loci covaried positively. Both subpopulation differentiation and the covariance of heterozygosities could result from selection acting on individual loci in concert but varying in intensity among an initially homogeneous set of subpopulations. Selection against heterozygotes which varies in intensity among subpopulations can produce large heterozygote deficiencies and MLH disequilibria, if the mean fitness of heterozygotes is substantially lower than that of homozygotes (Table 6A). It could also produce the correlation observed between the mean single-locus D of paired loci and their dilocus disequilibria, if selection against heterozygotes affected a suite of loci in parallel. When fitnesses of alleles vary among subpopulations and the less fit allele

TABLE 6

Selection varying among subpopulations as a source of heterozygote deficiencies and MLH disequilibria

A. Selection against heterozygotes. Viability of heterozygotes varies randomly from 0 to 1 among subpopulations and is identical for both loci in any given subpopulation. p_0, q_0, r_0 = initial allele frequencies.

Locus	p_0	q_0	r_0	D	D_H	D'_H
A	0.1	0.2	0.7	−0.23	0.022	0.142
B	0.3	0.4	0.3	−0.32		

B. Selection against the dominant allele. Viability of homozygotes varies randomly from 0 to 1 among subpopulations; in each, the favored allele is completely recessive. Fitness arrays same for both loci in any given subpopulation.

Locus	p_0	q_0	r_0	D	D_H	D'_H
A	0.1	0.2	0.7	−0.20	0.006	0.034
B	0.3	0.4	0.3	−0.20		

is dominant, modest single-locus deficiencies and MLH disequilibria result (Table 6B).

The latter model suffers from the constraints that dominance relationships among alleles are not fixed and genotypic fitness arrays covary among loci, but does not require mean fitnesses to differ among genotypes. Both models are difficult to test because of the inaccessibility of larval subpopulations to genetic sampling before they combine at settlement, and both require very large fitness differences among alleles.

As an alternative to models invoking strong selection, the mixing of subpopulations which differ in degree of aneuploidy or imprinting can generate both large heterozygote deficiencies and MLH disequilibrium. However, in order to produce allele-specific variation in heterozygote deficiencies, the subpopulations would have to differ in allele frequencies as well.

Heterozygosity, size and heterozygote deficiencies

There is one final observation to be explained, namely the growth-heterozygosity correlation and its relationship to heterozygote deficiencies. The presence of a correlation between SL_i and MLH requires two different models of mixing to be considered. One is that the sample of *M. lateralis* examined in this study represents more than one spatfall, with earlier-settling cohorts being on average more heterozygous. The alternative is simultaneous settlement of differentiated subpopulations, followed by MLH-dependent differences in growth rate. In the first case, those loci with the largest among-cohort differences in heterozygosity would show the largest variance in allele frequencies, thereby generating an association between the heterozygosity-size relationship and heterozygote deficiency. In the second case, the variance of allele frequencies among subpopulations is positively correlated with the locus-specific effects of heterozygosity on growth rate. We examine these hypotheses below.

Several observations are inconsistent with the hypothesis of asynchronous settlement. First, only one locus showed significant heterogeneity of allele frequencies among size deciles. Secondly, intermediate size classes should show the largest deficiencies, as they would be more likely to comprise several cohorts than the extreme size classes (*i.e.*, the earliest and latest settlers). This was not observed (Table 2). Finally, the similar relative contributions of individual loci to growth measured directly in the laboratory (KOEHN, DIEHL and SCOTT 1988) indicates that the association between size and heterozygosity is not merely a fortuitous consequence of differential settlement.

If size reflects cohort of origin, as would be required with sequential settlement, we might expect adjacent size classes to be genetically more similar to one another than non-adjacent ones. Genetic distance among deciles was positively correlated with interdecile interval ($F_{1,7} = 21.3, P < 0.005$). However, this relationship could also be obtained from simultaneous settlement with growth rate a function of heterozygosity, because the nonrandom distribution of heterozygosity with respect to size would lead to allele frequency differences among size classes.

In order to separate these two potential sources of a heterozygosity-size relationship we examined the distribution of allele frequencies in the homozygous subsets of size deciles. In a single cohort, allele frequencies in homozygotes are constant across size classes, even if heterozygotes grow faster and are therefore more frequent in the larger size classes. If on the other hand the sample comprises multiple subpopulations, allele frequencies in homozygotes will vary among size classes as a result of the differential contributions of the subpopulations to each size class.

Genetic distance based on allele frequencies in homozygotes was not correlated with interdecile interval ($F_{1,7} = 0.47$ NS). Therefore, the most plausible interpretation is that of a single spatfall in which the size-heterozygosity relationship is due primarily to MLH-dependent growth rather than differences in the time of settlement.

If our sample does indeed represent a single cohort in which heterozygote deficiencies are the result of combining differentiated subpopulations at the time of settlement, how can we account for the size-heterozygosity correlation and its relationship to heterozygote deficiencies? Again, both selectionist and nonselectionist hypotheses may be advanced.

Selection reversal: As noted earlier, both large heterozygote deficits and a positive growth-heterozygosity correlation could be obtained by a reversal of selection at the time of larval settlement, either (1)

from underdominance to overdominance, or (2) from recessiveness to dominance of the favorable allele. Neither is compatible with the notion that the allozymes are merely markers for associated deleterious alleles (*i.e.*, associative overdominance). Instead, it would be necessary for allozyme variants themselves to vary greatly in fitness, or for them to be in linkage disequilibrium with loci that undergo reversals of selection.

The heterogeneity of D values among and within loci is compatible with either selection against heterozygotes or selection against dominant alleles. The correlation between locus-specific heterozygote deficiencies and locus-specific effects of heterozygosity on size would occur if those loci strongly selected during larval life were also subject to strong selection after settlement, although in the opposite direction.

In order to maintain multiallelic polymorphisms, strong selection against heterozygotes during one stage of the life cycle must be counterbalanced by strong selection in their favor during another stage. We therefore might expect alleles which show large allele-specific heterozygote deficiencies (D_j) to show correspondingly large heterotic effects, *i.e.*, a negative relationship between D_j and mean SL_i of heterozygotes for the jth allele. This was the case for βGAL (6 alleles, $r = -0.949$, $P < 0.01$) and AP2 (4 alleles, $r = -0.966$, $P < 0.05$), but the reverse was true for AP1 (5 alleles, $r = 0.957$, $P < 0.05$) and AP3 (5 alleles, $r = 0.867$, $P < 0.05$).

Aneuploidy/imprinting: Without invoking fitness differences among alleles, either of these hypotheses can explain locus-specific heterozygote deficiencies, heterotic effects, and the correlation between them. The mixing of subpopulations which vary not only in degree of aneuploidy/imprinting but also in allele frequencies could account for MLH disequilibrium and the variation in heterozygote deficiencies among alleles within loci.

In conclusion, no single one of the hypotheses advanced to account for heterozygote deficiencies or the heterozygosity-size correlation can produce the patterns we have seen in *M. lateralis*. The coupling of subpopulation mixing with either strong selection, aneuploidy or molecular imprinting is sufficient to produce the observed patterns. These hypotheses are amenable to testing in the laboratory and should be further explored.

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