Fixation, Segregation and Linkage of Allozyme Loci in Inbred Families of the Pacific Oyster *Crassostrea gigas* (Thunberg): Implications for the Causes of Inbreeding Depression

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ABSTRACT

The effect that inbreeding has on the fixation and segregation of genes has rarely been confirmed by direct observation. Here, fixation, segregation, and linkage of allozymes is investigated in the progeny of self-fertilized hermaphrodites of the normally outcrossing Pacific oyster *Crassostrea gigas*. The estimate of fixation pooled over loci, individuals, and families, $F = 0.462$, is significantly lower than the expected value of 0.5. Log-likelihood ratios reveal significant heterogeneity in fixation among individuals, among families, and among loci. In addition, the grand pooled segregation ratio, 127:243:54, deviates significantly from 1:2:1, with a bias against homozygotes for alleles of lesser frequency in the natural population. Segregation ratios for 11 of 14 loci are significantly heterogeneous among families, and exact tests for segregation within families reveal 16 significant results out of 51 tests. Thus, fixation and segregation of allozyme markers in inbred oyster families deviates from the expectations of neutral inbreeding theory. Di-genic disequilibria are significant for four of 74 di-locus pairs revealing two linkage groups. Strong viability selection is apparently conditional on the genotype of the hermaphrodite-founders and is largely focused on these two linkage groups. These genetic effects are explained by interaction between *cis*-linked factors and polymorphic regulatory backgrounds.

POPULATION genetic study of marine molluscs, particularly bivalves, which have exceptionally large amounts of electrophoretically detectable protein variation (BUROKER *et al.* 1979; FUJIO *et al.* 1983; NEVO *et al.* 1984; BLANC and BONHOMME 1987), is germane to the general, still unresolved debate over the adaptive significance of this variation (LEWONTIN 1974). Allozyme heterozygosity has been directly related to traits associated with fitness for molluscs. There is, for example, a significant tendency for mean weight to be correlated with allozyme heterozygosity in cohorts of oysters derived from natural populations (SINGH and ZOUROS 1978; ZOUROS *et al.* 1980; FUJIO 1982). Moreover, allozyme heterozygosity is inversely correlated with routine respiration rate, providing a physiological rationale for the positive correlation between allozyme heterozygosity and growth rate (KOEHN and SHUMWAY 1982; GARTON *et al.* 1984; HAWKINS *et al.* 1989; HILBISH *et al.* 1994). Two hypotheses have emerged to explain these observations: the overdominance hypothesis, which posits a direct functional role for allozymes in fitness, and the associative overdominance hypothesis, which holds that allozymes, as genetic markers, are only indirectly associated with fitness-related traits through linkage disequilibria with agent loci segregating for deleterious recessive alleles (see reviews by ZOUROS and FOLTZ 1987; ZOUROS and POGSON 1994). Evidence in support of a direct role for allozymes includes the greater contributions to the growth-rate *vs.* heterozygosity correlation by enzymes involved in glycolysis and protein catabolism than by other proteins (KOEHN *et al.* 1988), overdominance at single allozyme loci (*cf.* GILLESPIE and LANGLEY 1974; KACSER and BURNS 1981; SUGITA and FUJIO 1982; POGSON 1991; SARVER *et al.* 1992), and correlation of protein markers but not restriction fragment length polymorphism (RFLP) DNA markers with growth (POGSON and ZOUROS 1994). Evidence in support of an indirect role for allozymes includes failure to find a correlation between allozyme heterozygosity and fitness-related traits in hatchery-produced cohorts (BEAUMONT *et al.* 1983; GAFFNEY and SCOTT 1984; BEAUMONT 1991) or in temporal samples from the same locality (GAFFNEY 1990), correlation between heterozygote-deficiency at an allozyme marker and that marker's contribution to the multiple-locus heterozygosity-fitness correlation (GAFFNEY *et al.* 1990), and relatively high frequencies for null alleles at allozyme loci (FOLTZ 1986; GAFFNEY 1994). However, these studies vary in at least three key ways that may obscure the true association of allozymes and fitness-related traits: (1) the specific loci examined are not the same across studies, which varies the chromosomal linkage relationships of the marker loci observed; (2) the population structures vary from cohorts to mass spawnings to pair crosses, which confounds the amount of gametic phase disequi-

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librium and the specific alleles segregating in the sample units; and (3) the species examined varies from snails, to oysters, clams, mussels, scallops etc., which confounds differences in ecological and selective regimes, chromosome number and structure, phylogeny, and evolutionary history. Given the many differences among studies, the extent to which they find heterozygosity-fitness correlation in bivalve molluscs is all the more remarkable.

We have adopted crosses among inbred progeny from selfed Pacific oysters as an experimental approach to testing alternative explanations for heterozygosity-fitness correlation and for heterosis in general (HEDGECOCK *et al.* 1995, 1996). This approach includes the production of inbred lines and the verification of parentage for the broodstock, because contamination among bivalve larval cultures is widespread (FOLTZ 1986; GAFFNEY and ALLEN 1993; MALLET *et al.* 1985; ZOUROS *et al.* 1992). Here we show that allozyme markers are sufficient to confirm the parentage of inbred families, and further, we estimate fixation rates, segregation ratios, and linkage of allozyme markers for seven inbred families. Data are analyzed in a hierarchical fashion, separating observations by locus within family, by individuals within family, by families within the collection of inbred lines, by locus across families, and over all individuals and loci (the grand pool). According to the laws of Mendelian inheritance and neutral inbreeding theory (WRIGHT 1917), inbred families should exhibit 1:2:1 segregation ratios for each allozyme locus heterozygous in the selfed hermaphrodite, making half of each family homozygous, identically by descent. We ask whether allozyme markers conform to these expectations, both on average and across individuals, families and loci.

MATERIALS AND METHODS

Experimental material: Six hermaphroditic oysters were selfed in 1989 by KEN COOPER of Coast Oyster Co. (now Coast Seafoods, Bellevue, WA) to produce lines 89-1, 89-3, 89-4, 89-5, 89-6, and 89-7. In addition, a hermaphrodite was self-fertilized at the Bodega Marine Laboratory in 1992 to produce line 92-1. These seven hermaphrodites, hereafter called founders, were all collected from the semi-isolated, naturalized population of *Crassostrea gigas* in Dabob Bay, Puget Sound, Washington, which was itself established by massive importation of seed oysters from native populations in Miyagi, Japan (CHEW 1979); tissue samples of founders were not kept and were thus unavailable for electrophoretic analysis. Full-sibs of families 89-1 and 89-5 were mated in 1992 to produce a second generation of inbred stocks, designated 92-89-1 and 92-89-5, respectively. Each family was caged separately and set out to grow in Tomales Bay, California, prior to crosses conducted in the summers of 1993 and 1994. Owing to small numbers, sampling was restricted to a total of 99 adult individuals that were destructively killed in the process of making second-generation experimental crosses (see HEDGECOCK *et al.* 1995, 1996). After sacrifice, brood stock (both parents of second-generation crosses and unmated sibs of the first inbred generation) were each stored at −80° in plastic bags labeled with the sex, family, and an individual number.

Allozyme electrophoresis: A survey of 24 loci (*Aat, Acon-1, Acon-2, Adk, Dia, G-3-pdh, Gpi, Dap-2, Idh-1, Idh-2, Lap-1, Lap-2, Tap-3, Mdh-1, Mdh-2, Mpi, 6-pgdh, Pgm, To-1, To-2, Pt-1, Pt-2, Mp-1,* and *Sdh*) yielded 14 loci that were segregating in at least one family and were thus inferred to be heterozygous in at least one of the seven founding hermaphrodites. Electrophoretic protocol and allozyme nomenclature were essentially that of HEDGECOCK and SLY (1990) and HEDGECOCK (1994), who together with BANKS *et al.* (1994) are the sources for allelic frequencies in the Dabob Bay population for all loci but *Sdh*; allelic frequencies for *Sdh* are here inferred from the founders.

Verification of pedigrees: Because only two alleles can be segregating in the progeny from a selfed diploid, the presence of more than two alleles at any locus is evidence of contamination of the full-sib family examined. Those individuals having the least frequent of the three alleles can be excluded; for example, if most sibs are *AA, AB,* or *BB* at a locus, a putative sib with genotype *AC* is assumed to be a contaminant and is excluded from further analysis. Genotypes of the founders were inferred from the genotypes of the nonexcluded progeny. Finally, the parentage of each progeny was ascertained on the basis of allozyme genotype, using a computer program written in the IML language of SAS (SAS Institute, Inc., 1988; D. J. MCGOLDRICK, unpublished data). This program performs, for each progeny genotype, a step-wise check, by locus, then family, for consistency with the genotypes expected from self fertilization of the founders, assuming Mendelian inheritance of markers. Failure to be excluded at any locus results in assignment of a progeny to that family. Ideally, each progeny would be assigned to one and only one family, the same family with which that individual had been caged and identified. To test how often a randomly drawn wild individual could match any of the seven inbred families by chance, we generated 1000 simulated multilocus genotypes by computer assisted, pseudomultinomial sampling from the known allele-frequency distributions in Dabob Bay. We assumed gametic phase equilibrium at the 14 loci, which was justified by the absence of significant digenic disequilibria in the Dabob Bay population, using contingency tables and a resampling program (D. ZAYKIN, personal communication). This random test population was treated as a putative progeny group and matched against sets of genotypes expected from the selfed founders.

Statistical analysis: Raw genotypic data for the 99 individuals are tabulated in the Appendix. We addressed fixation levels and segregation ratios separately. The fixation index, F, was calculated as $F = 1 - (HG1/HP)$, where $HG1$ and HP are the heterozygosities for the first inbred generation and the founder, respectively. To analyze segregation, we tabulated, by locus and across group (family or total), three pooled genotypic proportions, A_iA_i, A_iA_j, A_jA_j, where the frequency of allele A_i is greater than that of allele A_j in the Dabob Bay wild population. For nine loci, the A_i and A_j alleles were the same for all families; for three loci (*Acon-1, Adk, Sdh*), allele A_i was the same but allele A_j differed from family to family; for only two loci (*Aat, Pgm*), did both alleles vary across families. Segregation in the second-generation 92-89-5 group, which resulted from the mating of male 3 by female 4 of 89-5 (APPENDIX), was analyzed separately.

Hierarchical log-likelihood ratio or G-tests (SOKAL and ROHLF 1981) were made to assess both agreement with mean expectations ($F = 0.5$ or 1:2:1 segregation ratios, tested by G_{pooled} and G_{grand} statistics) and the heterogeneity of fixation levels or segregation ratios within and among families and loci (tested by $G_{individuals}$, $G_{families}$, and G_{loci} statistics). Significance levels of G-tests were adjusted for simultaneous testing over multiple families, individuals, or loci (WEIR 1990); however, the correction was not applied to tests of heterogeneity by

locus across families because loci proved significantly heterogeneous in fixation and segregation. Exact tests for individual segregation ratios within particular families were conducted (by summing over all outcomes with equal or lesser probability). These tests were not corrected for multiple testing because of significant family by locus interaction. In addition, we pooled genotypic classes for loci on two known linkage groups and tested for 1:2:1 pooled segregation and heterogeneity among loci within linkage groups.

Linkage disequilibrium and linkage: With 14 allozyme loci, 91 unique di-locus comparisons were possible. For each comparison, tests of association were based on the significance of a di-locus disequilibrium coefficient, which was estimated by a resampling procedure that tests for di-genic and higher order disequilibria (D. ZAYKIN, personal communication). Pairs of loci with significant associations in at least one family were then examined for evidence of linkage. To estimate linkage, we used the maximum likelihood approach, combining data from all families for which at least one parent was doubly heterozygous and segregation was Mendelian (see ALLARD 1956; KOROL *et al.* 1994). Portions of the data set were not used for estimating linkage because of deviations from 1:2:1 segregation. Families that failed Mendelian segregation tests were dropped from data sets used for maximum likelihood estimation. Standard deviations for the uncorrected recombination fraction were estimated by taking the inverse of the square root of the total information for a given di-locus pair. Finally, map distances were derived, correcting for interference with KOSAMBI's mapping function (KOSAMBI 1944; KOROL *et al.* 1994).

RESULTS

Contamination: Five of 99 progeny (5.0%) are classified as contaminants: males 16, 17, and 18 in family 92-89-1, female 1 in family 89-3, and female 4 in family 89-7 (APPENDIX). Whereas, in family 89-7, five sibs are *AI* and one is *AA* at the *Pgm* locus; female 4 in this family is heterozygous *Ab* (lower case letters denote third alleles within families). The genotype of this same female is unlikely, though not excludable, at three other loci (she is *AD* at *Aat* in a sibship of *AA*, *AE* at *Gpi* in a sibship of *AA*, and *FF* at *Lap-2* in a sibship of *AA*), further supporting classification as a contaminant. This female has a genotype that cannot occur in any of the seven inbred families. Regarding the four other contaminants detected, female 1 in family 89-3 is rejected by a third allele at *Adk*, males 17 and 18 of family 92-89-1 are rejected by third alleles at *Lap-2* and *Sdh*, while male 16 of this family is rejected by third alleles at *Aat*, *Adk*, and *Lap-2*. None of these individuals has a genotype consistent with any of the other inbred families.

Family assignments: Excluding these five contaminants, 94 remaining progeny have genotypes consistent with their family and founder. Based solely on allozyme genotype, 78 of these, 83%, are assigned to one and only one family, the same family with which each was caged and identified. Of the remaining progeny-genotypes, 14 (14.9%) are assigned to the expected family plus one other, and two (2.1%) are assigned to three families, including the expected family. Only one of the 1000 random-test genotypes fit a family by chance (and this genotype would have appeared 50% fixed). Conversely, 999 of 1000 random-test genotypes do not fit any family and would be rejected as contaminants. Since we actually reject five individuals, we estimate the number of undetected contaminant individuals as $5/999 = 0.005$. This yields an estimate of $0.005/99 \sim 0.00005$ for the probability that undetected contaminants remain in any one of the families.

Two-locus disequilibria and linkage: Of the 91 possible di-locus comparisons, only three fail to have at least one parent doubly heterozygous in at least one family, leaving 88 di-locus pairs as preliminary candidates for linkage tests. Excluding pairs that deviate from Mendelian segregation leaves 74 di-locus pairs. Of these, four pairs show significant di-locus disequilibria by the resampling test (Table 1A). Using the resampling test results as indicators of potential linkage, we estimate recombination fractions by pooling data for all informative families having Mendelian segregation for the di-locus pairs of interest, where a value of 0.5 is the expectation when there is no linkage (Table 1B). In addition to the four significant associations, we also estimate recombination fractions for two implied associations, *Aat/6-pgdh* and *Acon-1/Idh-2*. Two linkage groups are evident, one containing *Aat*, *Gpi*, and *6-pgdh* and a second with *Idh-2*, *Acon-1*, and *Pgm*. The most likely gene orders for these linkage groups (in Kosambi map units) are as follows: (1) *Aat*–28.8–*Gpi*–27.4–*6-pgdh* and (2) *Idh-2*–23.6–*Acon-1*–9.9–*Pgm*.

Average fixation levels: An estimate of the fixation index averaged over all inbred lines and loci is $F = 0.462$ (bottom rows of Tables 2 and 3). This grand pooled fixation estimate is slightly but significantly lower than the expected 0.5 ($G_{grand} = 4.099$, 1 d.f., $P = 0.043$). Moreover, there is significant heterogeneity in fixation levels both among families ($G_{families} = 72.395$, 6 d.f., $P \ll 0.001$, Table 2, bottom row) and among loci ($G_{loci} = 39.759$, 13 d.f., $P \ll 0.001$, Table 3, bottom row).

Fixation levels by family: We now consider, by family, *G*-tests of mean against expected fixation and of heterogeneity of fixation among loci and among individuals (Table 2) and find some families that are overfixed and some that are underfixed. Family 89-1 is significantly less fixed than neutral inbreeding theory would predict ($F = 0.217$; $G_{pooled} = 36.058$, 1 d.f., $P < 0.001$, Table 2). There is no significant heterogeneity in fixation among the 89-1 sibs or among the eight segregating loci. Family 89-4, on the other hand, is significantly overfixed ($F = 0.690$; $G_{pooled} = 14.809$, 1 d.f., $P \ll 0.001$); there is no significant heterogeneity among loci, but, after correcting for multiple testing, there is significant heterogeneity among sibs ($G_{individuals} = 23.554$, 9 d.f., $P = 0.035$). One sib is completely fixed for all 10 loci that were heterozygous in the parent, and three sibs are fixed for all but one locus while the remaining sibs fix as expected. Family 89-5 has no systematic deviation in the pooled fixation index and no heterogeneity of fixation among individuals; however, it does have significant heterogeneity of fixation among loci ($G_{loci} = 33.513$, 11

TABLE 1

Two-locus disequilibria (A) and linkage (B) of allozyme markers

Loci paired	*D(AB)*	*D(AAB)*	*D(ABB)*	*D(AABB)*	Family	Mendel?
	A. Significance of two-locus disequilibria coefficients					
Aat/Gpi	0.04625	0.04000	0.04219	0.02875	89-4	No
Gpi/6-pgdh	0.01656	0.00125	0.00094	NS	92-89-5	Yes
Acon-1/Pgm	0.03562	0.01438	NS	NS	92-89-5	No
Idh-2/Pgm	0.02937	0.73313	0.49656	0.81094	89-3	Yes

Loci paired	Families used	Recombination fraction[a]	Kosambi map units (cM)
	B. Linkage		
Aat/Gpi	89-5, 6	0.25981 ± 0.1077	28.8
Gpi/6-pgdh	89-5, 92-89-5	0.24858 ± 0.0741	27.4
Aat/6-pgdh	89-5	0.34277 ± 0.1954	42.0
Acon-1/Pgm	89-3, 5, 7, 92-1	0.09979 ± 0.0432	9.9
Acon-1/Idh-2	89-3, 92-1	0.21960 ± 0.0851	23.6
Idh-2/Pgm	89-3, 92-1	0.24595 ± 0.0832	26.9

[a] Values are means ± SD. NS, not significant.

d.f., $P = 0.003$). *Lap-2* and *Pgm* are underfixed in this family, while *Sdh* and *Dap-2* are overfixed. Family 89-6 is significantly underfixed ($F = 0.129$; $G_{pooled} = 19.133$, 1 d.f., $P < 0.001$) but has no significant heterogeneity either among loci or sibs. The remaining three families, 89-3, 89-7, and 92-1, have mean fixation levels no different than expected and no heterogeneity of fixation, either among individuals or loci.

Fixation levels by locus: We next examine fixation by locus, over families (Table 3, for loci grouped into two linkage groups, shown in A and B, and unlinked markers, in C), classifying departures from neutral inbreeding expectations as systematic, when mean fixation for a locus across families is not 0.5 (tested by G_{pooled}), and erratic, when there is significant heterogeneity of fixation among families for a locus (tested by $G_{families}$). We conservatively treat each locus as representing an independent test of the same fixation hypothesis and therefore adjust the significance of the G_{pooled} statistic for multiple testing. However, because there is substantial heterogeneity in fixation among loci (G_{loci}, bottom row, Table 3), we consider the test of heterogeneity among families for each locus to be a unique test, with degrees of freedom equal to one less than the number of families in which the locus is segregating. Probabilities associated with the systematic and erratic departures from expected fixation for each locus are plotted in Figure 1. The magnitude of the heterogeneity in fixation among families is colinear with the estimated gene orders for *Aat, Gpi,* and *6-pgdh* as well as *Idh-2, Acon-1,* and *Pgm.*

Seven of 14 segregating loci (*Acon-2, Adk, Dia, G-3-pdh, Dap-2, Idh-2,* and *Mp-1*) show neither systematic nor erratic departures from neutral inbreeding expectations (Table 3 and Figure 1). Of the remaining seven loci, however, *Lap-2* has a significant excess of heterozy-

TABLE 2

Fixation of allozyme markers within inbred families of Pacific oysters

Family	No. of loci	No. of individuals	No. of heterozygotes		Estimated fixation[a]	*G*-tests for *F* heterogeneity		*G*-tests, mean *F*
			Expected	Observed	*F*	G_{loci}	G_{indiv}	G_{pooled}
89-1	8	15	53	83	0.217 ± 0.040	16.538	22.140	36.058***
89-3	6	11	33	42	0.364 ± 0.059	3.306	20.619	4.972
89-4	10	10	50	31	0.690 ± 0.046	22.479	23.554*	14.809***
89-5	12	38	173.5	167	0.519 ± 0.027	33.513**	46.580	0.487
89-6	4	8	15.5	27	0.129 ± 0.060	0.015	12.751	19.133***
89-7	6	7	18.5	18	0.514 ± 0.082	7.101	7.356	0.027
92-1	5	5	12.5	15	0.400 ± 0.098	1.726	3.452	1.007
						$G_{families}$		G_{grand}
Pooled	14	94	356	383	0.462 ± 0.019	72.395***		4.099*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] Values are means ± SD.

TABLE 3

Fixation of allozyme loci among inbred families of Pacific oysters

Locus	No. of families	No. of heterozygotes		Estimated fixation[a]	G-test for F heterogeneity	G-tests, mean F
		Expected	Observed	F	$G_{families}$	G_{pooled}
			A. Linkage group I			
Aat	4	31.5	31	0.508 ± 0.063	9.103*	0.016
Gpi	3	24	24	0.520 ± 0.071	8.962*	0.080
6-pgdh	3	31.5	32	0.492 ± 0.063	18.237***	0.016
			B. Linkage group II			
Idh-2	4	19.5	28	0.282 ± 0.072	3.472	7.665
Acom-1	5	32.5	29	0.554 ± 0.062	9.872*	0.755
Pgm	5	35	43	0.386 ± 0.058	15.553**	3.690
			C. Unlinked			
Lap-2	4	34	48	0.294 ± 0.055	17.713***	11.880**
Mp-1	5	21	29	0.310 ± 0.071	3.328	6.252
Sdh	4	18	15	0.583 ± 0.082	19.865***	1.005
Acon-2	3	22.5	21	0.533 ± 0.074	0.404	0.200
Adk	3	32	41	0.359 ± 0.060	3.506	5.131
Dap-2	3	25	16	0.680 ± 0.066	5.128	6.628
Dia	3	16	14	0.563 ± 0.088	1.834	0.501
G-3-pdh	2	12.5	12	0.520 ± 0.100	0.338	0.040
					G_{loci}	G_{grand}
Pooled	7	356	383	0.462 ± 0.019	39.759***	4.099*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] Values are means ± SD.

gotes ($F = 0.294$), and all seven show erratic deviations in fixation among families. Systematic departures from expected fixation are large for *Adk, Mp-1, Dap-2,* and *Idh-2,* but these fail to achieve significance after correcting for multiple testing. Systematic and erratic departures appear uncorrelated; heterogeneity in fixation is significant for *6-pgdh, Gpi,* and *Aat,* which have low systematic deviations, as well as for *Sdh, Lap-2, Pgm,* and *Acon-1,* which have larger systematic departures. Significant heterogeneity for fixation of linked allozyme loci (*Aat-6-pgdh-Gpi* and *Acon-1-Pgm*) is accompanied by concordance of fixation levels for these markers within families, whether over or under the expected value of 0.5 (see bottom of next section and Table 4).

Significant heterogeneity in fixation among families (Table 2) is not determined only by the systematic effects of allozyme loci that are segregating within a family. This observation is made clear by contrasting how loci are contributing to overall fixation in the two families that are overfixed (*i.e.,* losing too much heterozygosity) and the one that is underfixed (*i.e.,* retaining heterozygosity). The two families that are significantly underfixed, 89-1 and 89-6, are indeed segregating for some loci that tend to be systematically underfixed (*Adk, Mp-1, Idh-2,* and *Lap-2*). These loci surely contribute to the overall significance of the reduction in fixation in the family. However, in family 89-1, 7 of 12 progeny are heterozygous for *Dap-2,* a locus that tends to be overfixed. Similarly, the one family that is significantly overfixed, 89-4, is segregating for 10 loci, including 3 that meet expectation, 5 that are erratically fixed, and *Lap-2,* the only locus that is significantly underfixed on average. The three that meet expectation do not contribute much. The five erratic loci all show deficiencies of heterozygotes while at *Lap-2,* the locus that shows systematic underfixing, only 3 of 10, 89-4 progeny are heterozygous.

Segregation: To test whether segregation ratios for allozymes markers fit the 1:2:1 ratio expected from selfing, we pool genotypes into three classes, A_iA_i, A_iA_j, A_jA_j, where A_i represents (in 49 of 51 cases) the most frequent allele at a locus, and A_j comprises less frequent alleles in the Dabob Bay wild population, from which the hermaphrodite-founders were taken. The mean frequency of the lower ranked allele at these 14 markers in the Dabob Bay population is 0.123 (Figure 3), so the A_j are not necessarily rare, ranging in frequency from <0.001 to 0.358 with a median of 0.083 (*cf.* APPENDIX with data in HEDGECOCK and SLY, 1990; BANKS *et al.,* 1994; HEDGECOCK, 1994). Over all loci and families, the grand pooled segregation ratio, 127:243:54, deviates significantly from 1:2:1 (G_{grand} = 39.396, 2 d.f., $P \ll$

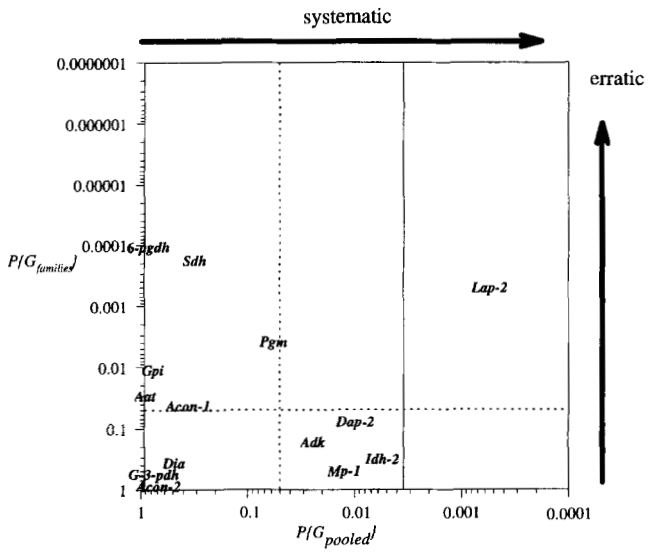

FIGURE 1.—Fixation of loci over families, presented as a bivariate plot of probabilities in *G*-tests for systematic ($P[G_{pooled}]$) and erratic ($P[G_{families}]$) departures from expected fixation of 0.5. The dotted lines indicate nominal significance levels, the solid line indicates an experiment-wide significance threshold for G_{pooled} corrected for 14 tests (0.00366). Several loci are significantly erratic in fixation while *Lap-2* shows systematic underfixing, with a significant erratic fixation response over families. The magnitude of erratic responses is colinear with the known gene orders for *6-pgdh, Gpi,* and *Aat,* as well as *Pgm, Acon-1,* and *Idh-2* (see Table 1).

0.001), with an overall bias against homozygotes for the allele of lesser frequency in the natural population (Table 4). Indeed, the grand pooled numbers of homozygotes for the common allele and heterozygotes are in a ratio, 127:243, that is not significantly different than 1:2 (goodness-of-fit $\chi^2 = 0.16$, 1 d.f.), suggesting that the deviation from the expected pooled segregation ratio of 1:2:1 results from a pervasive deficiency of homozygotes for less frequent alleles. Segregation ratios are significantly heterogeneous among the seven first-generation inbred families ($G_{families} = 100.835$, 12 d.f., $P \ll 0.001$). Five of the seven families (89-1, 89-3, 89-4, 89-5, and 89-6; see Table 4) have significant departures from the expected 1:2:1 ratio. Families 89-1 and 89-6, which are significantly underfixed (Table 2), show corresponding, substantial excesses of heterozygotes. Family 89-4, which is significantly overfixed, has the most extreme bias of any family towards homozygotes for the common allele.

Analyses of segregation ratios by locus, pooled over families, reveal significant departures, either systematic, erratic, or both, for 11 of 14 loci (Table 4). We consider each locus to represent a unique test of a segregation hypothesis because loci are segregating for different alleles and may form different multilocus associations; thus, the significance levels of the G_{pooled} tests are the nominal, $\alpha = 0.05$ level in Table 4 and Figure 2. Systematic and erratic departures from the expected 1:2:1 segregation ratio at each locus are more pronounced than the deviations from expected 0.5 fixation (*cf.* Figures 1 and 2). Exact tests for segregation within families that are significant at or above the $P = 0.05$ threshold are indicated in bold, and the significance levels of *G*-tests for heterogeneity of segregation are noted with asterisks (Table 4). As for fixation, we can infer that heterogeneity in segregation ratios across families is not solely caused by the particular loci segregating in families. Indeed, significant effects at a locus in a particular family can run counter to the overall trends for that locus; for example, *Gpi* is heterozygous in seven of eight 89-6 progeny despite an overall segregation ratio of 11:11:1 across a total of three families. Genotypic ratios in the second-generation family, 92-89-5, are also significantly different from Mendelian expectations for 4 of 11 sufficiently sampled segregating loci (*Pgm, Acon-1, Lap-2,* and *Dap-2*; Table 5).

Taking into account chromosomal linkage, we find that reductions in the homozygote for the rarer allele occurs globally in the pooled ratio, on both linkage groups, and for the third unassigned group, being (35:48:6) in the linkage group containing *Aat,* and (32:71:11) on the linkage group containing *Idh-2,* and (60:124:37) for the set of unassigned markers. Testing 1:2:1 segregation ratios within linkage groups reveals that three of four families fail systematically for one linkage group, and two of seven families for the other—or 5 of 14 total unique tests that fail (Table 4). There is no heterogeneity for the segregation of allozyme markers within the linkage group *Aat-Gpi-6-pgdh,* or *Idh-2-Acon-1-Pgm* (Table 4).

DISCUSSION

We have four major observations in these inbred progenies that must be explained: (1) Fixation and segregation of allozymes after inbreeding are distorted from neutral expectations. (2) Overall, there is a slight excess of heterozygotes, but fixation and segregation are extremely heterogeneous among families and loci. (3) The majority of significant effects are accounted for by two linkage groups and the magnitude of the effect appears colinear with the most probable gene order. (4) We observe very strong selection against rarer allozyme alleles. These observations cannot be attributed to contamination or lack of statistical power.

Deviations from the expected Mendelian segregation of allozymes are not uncommon in bivalves, having been reported in random pair-crosses of American oysters *C. virginica* (FOLTZ 1986; HU *et al.* 1993), Pacific oysters (THIRIOT-QUIEVREUX *et al.* 1992), and the mussel *Mytilus edulis* (BEAUMONT *et al.* 1983). In our study, the pervasive deficiency of homozygotes for rarer alleles implies strong selection. If a genotypic ratio of 127:243:54 were observed in the first generation after selfing at a single locus with complete dominance, a reduction in the frequency of the recessive allele as large as that observed for the pooled A_j allele, from 0.5 to 0.4, would imply a relative fitness for the rare

TABLE 4

Segregation of allozyme loci in inbred families of the Pacific oyster

	Family																					Marginal		
	89-1			89-3			89-4			89-5			89-6			89-7			92-1					
A. Linkage group I																								
Aat	4	7	2	—	—	—	**8**	**2**	**0**	2	3	0	1	7	0	—	—	—	—	—	—	**15**	**19**	**2***
Gpi	—	—	—	—	—	—	**8**	**2**	**0**	2	2	1	1	7	0	—	—	—	—	—	—	**11**	**11**	**1***
6-pgdh	**0**	**14**	**1**	—	—	—	**8**	**2**	**0**	1	2	2	—	—	—	—	—	—	—	—	—	9	18	**3*****
Pooled	**4**	**21**	**3**	—	—	—	**24**	**6**	**0**	5	7	3	**2**	**14**	**0**	—	—	—	—	—	—	**35**	**48**	**6*****
B. Linkage group II																								
Idh-2	**3**	**12**	**0**	1	6	4	—	—	—	—	—	—	1	7	0	—	—	—	0	3	2	**5**	**28**	**6***
Acon-1	—	—	—	3	8	0	**6**	**4**	**0**	0	4	1	—	—	—	2	5	0	1	3	1	**12**	**24**	**2****
Pgm	—	—	—	3	6	2	**9**	**1**	**0**	2	3	0	—	—	—	1	5	0	0	4	1	**15**	**19**	**3*****
Pooled	**3**	**12**	**0**	7	20	6	**15**	**5**	**0**	2	7	1	1	7	0	3	10	0	1	10	4	**32**	**71**	**11*****
C. Unlinked																								
Lap-2	**0**	**15**	**0**	—	—	—	**7**	**3**	**0**	**0**	**3**	**2**	—	—	—	—	—	—	2	3	0	**9**	**24**	**2*****
Mp-1	2	7	0	4	7	0	0	7	3	—	—	—	0	6	1	3	2	0	—	—	—	**9**	**29**	**4*****
Sdh	0	10	2	—	—	—	**8**	**0**	**2**	**4**	**1**	**0**	—	—	—	0	2	4	—	—	—	12	13	**8*****
Acon-2	—	—	—	—	—	—	**6**	**4**	**0**	3	2	0	—	—	—	—	—	—	0	2	3	9	8	**3****
Adk	1	11	**3**	2	9	0	—	—	—	**4**	**1**	**0**	—	—	—	—	—	—	—	—	—	7	21	**3****
Dap-2	2	7	3	—	—	—	—	—	—	**0**	**1**	**4**	—	—	—	1	2	3	—	—	—	3	10	10
Dia	—	—	—	—	—	—	3	6	1	1	3	0	—	—	—	2	2	3	—	—	—	6	11	4
G-3-pdh	—	—	—	2	6	3	—	—	—	3	2	0	—	—	—	—	—	—	—	—	—	5	8	3
Pooled	**5**	**50**	**8***	8	22	3	**27**	**17**	**6*****	**15**	**11**	**8*****	0	6	1	6	8	10	2	5	3	**60**	**124**	**37*****
Grand	**12**	**83**	**11***	**15**	**42**	**9**	**63**	**31**	**6*****	**22**	**27**	**10***	**3**	**27**	**1**	9	18	10	3	15	7	**127**	**243**	**54*****

Boldface in the body of the table indicates $P < 0.05$ for an exact test of the segregation ratio. Asterisks denote significant heterogeneity of the segregation ratio among families using a *G*-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) Bolding in the margin indicates $P < 0.05$ for a *G*-test of the pooled segregation ratio.

homozygote only one-third that of the dominant phenotype (FALCONER 1989). The tendency to observe selection biases against rarer alleles in pair crosses is also not exclusive to this study. For example, deficiencies of homozygotes for the rarer esterase allele, (*Est-D*^{121}) in *M. edulis* have been observed (BEAUMONT *et al.* 1983), and the authors hypothesized that the bias was attributable to unobserved linked deleterious agents. In loblolly pines, "severe survival selection against rare alleles" was similarly observed for both selfed and outcrossed progeny (BUSH and SMOUSE 1991), suggesting a taxonomic generality to the observations that have been made of bivalve molluscs.

The rarer allozyme alleles have frequencies in the natural oyster population that are orders of magnitude greater than the simple mutation-selection equilibrium frequency for recessive alleles generated at typical rates of mutation (CROW and KIMURA 1970). How do these rare alleles persist if they are apparently so strongly selected against when homozygous? This "rare allele" paradox implies that more complex forms of balancing selection are at play both in the natural population and in the inbred families. We therefore reject purifying selection directed against rare allozymes as a general mode of selection in our families.

Heterogeneity in fixation levels among families for *Aat, Acon-1, Gpi, Lap-2, 6-pgdh, Pgm,* and *Sdh* (Table 3) and in segregation ratios among families and markers (Table 4) suggests that selection is not operating directly on the allozyme markers themselves. HU *et al.* (1993) observed a heterogeneous overdominant pattern in segregation ratios for *Gpi* in inbred families of *C. virginica* and concluded that *Gpi* is linked to a recessive gene of major effect. Our results confirm this heterogeneity in the congener, *C. gigas,* and associate it with distortions at the *Aat* and *6-Pgdh* loci (Figures 1 and 2 and below).

Linkage of allozymes has also been found in other studies. The linkage group containing *Aat-Gpi-6-pgdh* has been observed in families of *C. gigas* (G. POGSON, personal communication; X. GUO, personal communication) and in *C. virginica* (FOLTZ 1986). The second linkage group *Idh-2-Acon-1-Pgm,* has not been reported previously. We note that the majority of significant systematic and erratic departures from expected fixation and segregation are distributed on two linkage groups, accounting for five of seven significant tests for heterogeneity of marker fixation across families, five of seven significant tests for systematic departures from the 1:2:1 segregation ratio, and 6 of 11 significant tests for heterogeneity of segregation ratios across families. The significance thresholds of erratic effects appear to be co-

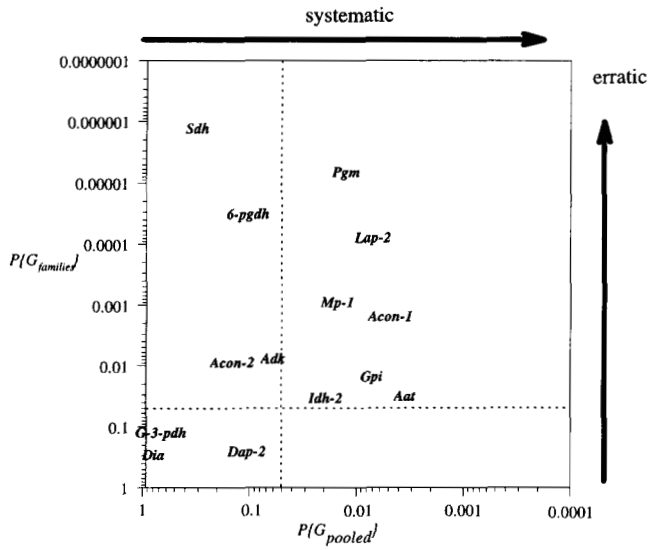

FIGURE 2.—Segregation, by locus and over families, presented as a bivariate plot of probabilities in *G*-tests for systematic ($P\{G_{pooled}\}$) and erratic ($P\{G_{families}\}$) departures from expected segregation of 1:2:1. The dotted lines indicate significant levels for a single *G*-test. Several loci deviate significantly erratically and systematically in segregation. The magnitude of erratic responses appears colinear with most probable gene orders for *6-pgdh*, *Gpi*, and *Aat*, as well as *Pgm*, *Acon-1*, and *Idh-2* (Table 1).

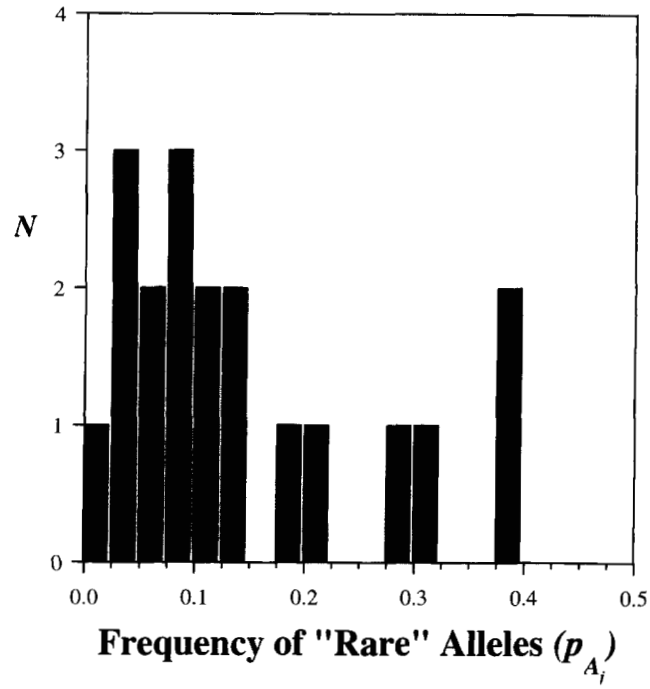

FIGURE 3.—Histogram of the frequencies of the *Aj* alleles (p_{Aj}) (from HEDGECOCK and SLY 1990; BANKS *et al.* 1994; HEDGECOCK 1994). The lower ranked A_j alleles are not necessarily rare; 10 of 19 have frequencies > 0.1, with a maximum frequency of 0.358 for the allele *Dap-2A*.

linear with the known gene order (Figures 1 and 2), but this may be coincidence. More linkage data are needed to map the chromosomal distribution of erratic fixation and segregation effects in the Pacific oyster.

Selection in inbred families of oysters: Most of these families (*e.g.*, all 89 lines) were created in the same spawning season and experienced common environments, so that genotype by environment interaction seems an unlikely explanation for the observed genotype-specific mortality. Contamination among cultures of bivalve larvae has been observed by several laboratories using genetic markers in mating experiments (MALLET *et al.* 1985; FOLTZ 1986; ZOUROS *et al.* 1992; GAFFNEY and ALLEN 1993), but these contaminants could be identified and excluded here. Rather than environmental effects, or contamination, the erratic fluctuations in fixation among families and markers apparently implicate selection against recombinant progeny genotypes, *i.e.*, segregational load.

Direct effects: Our observations invite comparisons with cases for which direct selection on allozyme polymorphism has been reported. For example, KOEHN *et al.* (1988) hypothesize that enzymes of glycolysis and protein catabolic function are more likely than others to be associated with the heterozygosity-growth correlation in the coot clam *Mulinia lateralis*. For the Pacific oyster, however, significant systematic or erratic departures from expected fixation and segregation are not confined to the 10 or 11 allozymes involved in amino acid or carbohydrate metabolism, but extend to proteins clearly outside of this group (*Adk*, *Mp-1*). On the other hand, *Lap-2* heterozygotes do appear to be systematically favored, and *Lap-2* has a protein catabolic role, which is consistent with KOEHN *et al.*'s hypothesis and with observations that differences in protein turnover account for much of the difference in the energy metabolism of slow- and fast-growing bivalve genotypes (HAWKINS *et al.* 1989; HEDGECOCK *et al.* 1996; J. VAVRA and D. MANAHAN, personal communication). Interestingly, SARVER *et al.* (1992) report apparent overdominance of specific activity for *Lap-2* in *C. virginica*, although they acknowledge that these results could be explained by closely linked modifier genes or non-Mendelian factors.

Finally, BUROKER (1979) suggests, on the basis of differential "survival" of genotypes between year classes of oysters reared at a tidal level of +1.2 m, that overdominance maintains the di-allelic polymorphism for muscle protein (*Mp-1*) in *C. gigas*. Although alternative explanations of allele-frequency change between years and tidal levels are possible for this case (HEDGECOCK 1994), *Mp-1* in our study does show a significant, though significantly heterogeneous excess of heterozygotes across five inbred families (9:29:4, Table 4). However, the fixation index for this locus, $F = 0.310$, does not depart significantly from the expected $F = 0.5$, when corrected for multiple testing (Table 3).

Thus, while some loci may indeed have direct effects on fitness, direct effects on all allozyme loci would not explain the heterogeneity of these deviations from fixation and segregation across families, *i.e.*, the conditioning of selective effects on founder genotype. Although

one might hypothesize that conditioning on founder genotype merely reflects the heterogeneity of allozyme alleles at the level of amino acid or DNA sequence, selection of the observed magnitude should rapidly eliminate deleterious alleles from the outbred population and reduce the extant allozyme polymorphisms to just those alleles maintained by balancing selection. Moreover, direct effects do not explain the rare-allele effect.

Epistasis and association: Linkage of *Aat, 6-pgdh,* and *Gpi* in *C. gigas* leads us to expect that strong selection on any one of these loci might affect deviations in the fixation and segregation of the others, which appears to be the case (Figures 1 and 2). In *Drosophila melanogaster, 6-pgdh* and *G-6-pdh* interact epistatically *in vivo* (EANES 1984); the interaction leads to high levels of 6-phosphogluconate, which then inhibits *Gpi* (KAHANA *et al.* 1960; KOEHN *et al.* 1983). If this interaction of *6-pgdh* and *G-6-pdh* occurs in cupped oysters and modulates 6-phosphogluconate levels, the fitness consequence of which (through inhibition of glycolysis) is further modulated by variants at the *Gpi* locus, the opportunity for coevolution of *Gpi* and *6-pgdh* alleles exists. Allelic variants might then be maintained in disequilibrium owing to linkage of *Gpi* and *6-pgdh* in *C. gigas* and *C. virginica.* Perhaps, the Drosophila epistatic selection scenario is occurring in oysters, or bivalves in general, and is exhibited as growth and survival differences for the whole linkage group (FOLTZ 1986), including *Gpi* (HU *et al.* 1993), *Aat* (see SUGITA and FUJIO 1982), and *6-pgdh.* Even so, this specific epistatic interaction would not solely account for our observations because allozymes on chromosomes without the *6-pgdh* and *Gpi* loci had distorted segregation ratios, heterogeneity of fixation and segregation across families, and selection against homozygotes for rarer alleles.

The pervasive distortions of fixation and segregation observed for allozyme loci could be explained by close linkage of these markers with a rather small number of genes having major effects on fitness. Evidence from oysters (FOLTZ 1986) and other bivalve species (BEAUMONT 1994) leads us to suspect that certain specific linkage relationships for allozymes might be detected in further investigations. If syntenic relationships hold, and no chromosomal fissions, or translocation have occurred, *Aat, Gpi, 6-pgdh, Xdh, Lap-2, EsD, Mpi,* and *Odh* may be on the same linkage group (BEAUMONT 1994) and hence may behave in correlated fashion in inbred families.

Lap, which is reported to be on the same linkage group as *Gpi* in the mussel *Mytilus edulis* (BEAUMONT 1994), has significant systematic and erratic departures from both expected fixation and segregation. It is possible, for example, that balancing selection at or near the *Lap-2* and *Mp-1* loci and epistasis associated with *6-pgdh* could explain most of our results. However, it is difficult to see how strong selection focused on a few agent loci or epistatic interactions could account for the rare-allele paradox. Further mapping of allozyme markers in large families is needed to resolve this issue.

Two classes of hypotheses appear to be capable of explaining all observations concerning fixation, segregation, and linkage of allozymes in inbred families of oysters. First are hypotheses that involve fitness genes distinct from, but linked to, allozyme loci. Second are hypotheses that involve the interactions of allozyme genes (including their promoters, signal sequences, and gene products) in various genetic backgrounds.

TABLE 5

Segregation of 11 allozymes in inbred Pacific oyster family, 92-89-5

Locus	Parental genotype		Observed nos.			Expected nos.			N	χ^2
	Male 3	Female 4	A_iA_i	A_iA_j	A_jA_j	A_iA_i	A_iA_j	A_jA_j		
				A. Linkage group I						
Aat	AD	AA	15	12	—	13.5	13.5	—	27	0.333
Gpi	EE	AE	14	13	—	13.5	13.5	—	27	0.037
6-pgdh	AA	AC	19	14	—	16.5	16.5	—	33	0.758
				B. Linkage group II						
Acon-1	AC	AC	21	5	1	6.75	13.5	6.75	27	40.333***
Pgm	AB	AA	9	24	—	16.5	16.5	—	33	6.818**
				C. Unlinked						
Lap-2	AC	CC	9	24	—	16.5	16.5	—	33	6.818**
Acon-2	AB	AA	12	13	—	12.5	12.5	—	25	0.040
Adk	AA	AC	13	20	—	16.5	16.5	—	33	1.485
Dap-2	AA	AB	21	6	—	13.5	13.5	—	27	8.333*
Dia	AD	AD	2	3	6	2.75	5.5	2.75	11	5.182
G-3-pdh	AB	AB	5	4	0	2.25	4.5	2.25	9	5.667

Family 92-89-5 is a second inbred generation made by mating full-siblings, male 3 and female 4 of family 89-5.

Associative overdominance: The associative overdominance hypothesis attempts to explain correlation of allozyme heterogeneity and fitness traits by linkage of the allozyme to fitness genes having deleterious recessive alleles. In our inbred families, the associative overdominance hypothesis requires deleterious recessive mutations to be preferentially associated in *cis* configuration with rarer allozyme alleles in the natural population (see ZOUROS 1993) because selfing of sampled heterozygotes produces segregation ratios that are systematically deficient for the rarer homozygote classes. The hypothesis offers no explanation for why recessive fitness mutations are repeatedly and preferentially in *cis* configuration with rarer allozyme alleles. Random mutations to deleterious recessive alleles at fitness loci should arise more often in *cis* configuration with more common alleles; instead only two of 14 loci (*Dap-2* and *Idh-2*) show a bias against homozygotes for the more common allele (Table 4). Therefore, it is necessary to explain how deleterious recessive mutations became preferentially associated with rarer allozyme alleles in the natural population of oysters in Dabob Bay. Associative overdominance cannot explain this rare allele paradox nor the patterns underlying the heterogeneity of fixation and segregation among families. An alternative hypothesis, however, can explain the rare allele paradox and the heterogeneity in the fixation and segregation ratios of our inbred lines.

Regulatory epistasis: Let us suppose that selection is operating on a hierarchically structured, epistatic gene regulatory system (*sensu* HEDRICK and MCDONALD 1980). At the base of the hierarchy are "producer genes," such as allozymes. At the top of this hierarchy are regulatory factors that modulate the expression of these "producer loci." We do not suppose these regulatory factors are necessarily genes, but at least the result of gene action, such as substrate that accumulates during metabolism (*e.g.*, 6-phosphogluconate) or part of the structure of cells (*e.g.*, molecular chaperones or membrane receptors). In these inbred lines, we suspect that responsive elements are linked in *cis* configuration with allozyme alleles and are coadapted with these regulatory factors thus forming an interaction system (*sensu* WRIGHT 1969).

One example of such genetic elements would be promoters (of allozyme loci or promoters of genes closely linked in *cis* configuration to allozymes). We envision variation in promoter sequence that is linked to neutral structural differences in the products whose transcription the promoters control (see PAIGEN 1989). We propose that rarer allozyme alleles are correlated with rarer promoter structures due to chromosomal linkage and the evolution of a multilocus systems. These promoters must be conditionally deleterious depending on genetic background and would persist in *cis* configuration for long periods of time. For example, if the recombination fraction were 0.001 cM, gametic disequilibrium in the gene complex would only be reduced by half in 693 generations (years) and would still be 0.05 in 2994 years (LEWONTIN 1974), if recombination is not suppressed and there is no selection. The frequency bias for coadapted promoter-gene complexes is maintained indefinitely by selection in a randomly mating population in the absence of stochastic forces.

Selective bias against homozygotes for rare alleles, under this hypothesis, is explained by a higher frequency of regulatory genetic backgrounds favoring A_i-linked promoters. The system could exhibit an apparent intermediate A_iA_j heterozygote in a *ii*-favoring regulatory genetic background and an overdominant A_iA_j in an *ij* background, while the A_jA_j preferring background *jj* remains rare. Indeed, such dominance fluctuation across families was significant at unlinked allozyme loci *6-Pgdh, Lap-2,* and *Adk* (Table 4). Family 89-1 is consistent with an *ij* genetic background, while family 89-4 is consistent with an *ii* genetic background. Furthermore, the fact that these three allozyme markers are unlinked suggests that chromosomal linkage might not be required to produce heterogeneity in the segregation and fixation of allozymes.

A further prediction under regulatory epistasis is that producer genes would exhibit greater segregational biases due to selection than other classes of genetic markers that do not interact with regulatory factors. Greater heterozygosity-growth correlation for protein markers rather than for RFLPs (POGSON and ZOUROS 1994) in *Placopecten magellanicus* (the deep-sea scallop) has been observed, but the authors concede that further data from diverse taxa is still needed before we can conclude that this is a general pattern.

The regulatory epistasis hypothesis also predicts a relationship between the magnitude of the fitness reduction and the measures of gene expression in cells, *e.g.,* specific messenger RNAs (mRNAs), allozyme amounts, or general protein content. This hypothesis is also congruent with the physiological observation that differences in protein turnover can account for much of the difference in the metabolism of slow- and fast-growing bivalve genotypes (HAWKINS *et al.* 1989; BAYNE and HAWKINS 1995; HEDGECOCK *et al.* 1996). Under this hypothesis, the regulation of genome expression would give rise to changes in levels of transcription of proteins, including allozymes, which would then influence components of physiological energetics such as protein metabolism, (DAMERVAL *et al.* 1994), oxygen uptake in adults and larvae (KOEHN and SHUMWAY 1982), and perhaps cellular stress response (J. S. CLEGG, personal communication), so as to produce metabolic load upon inbreeding, owing to loss of regulatory interactions. Other hypotheses could also produce such effects, *e.g.,* protein localization signals might be variable and interact with protein translocation systems (SCHATZ and DOBBERSTEIN 1996).

Thus, a regulatory-epistasis hypothesis can explain all of the features of our data and makes several specific predictions. Many observable quantities (mRNA expres-

sion differences, structural differences in promoters, protein expression differences, and higher correlation between allozyme markers and fitness-related responses) can be measured and used to falsify a null hypothesis of regulatory-epistasis.

Conclusions: Clearly, this study requires more replication. Studies of more families, with various levels of inbreeding, should be used to characterize fixation and segregation in Pacific oysters and other bivalves. Use of other DNA markers such as complementary DNA RFLPs (POGSON and ZOUROS 1994), anonymous single-copy nuclear DNA (KARL and AVISE 1993), EPIC PCR (PALUMBI and BAKER 1994), and microsatellites (WEBER and MAY 1989, MCGOLDRICK and HEDGECOCK 1996) will be beneficial for teasing apart the effects of direct selection on protein- or enzyme-coding genes from the effects of indirect selection on unobserved linked loci. Saturation of the genome with multiple markers will help to define how and where selection distorts fixation and segregation of different marker classes.

Two of the three major shortcomings of previous studies are alleviated in this study. We avoided variation in ecological and evolutionary histories by working within a deme, in common environments, and by using a common ancestral stock. We addressed variation in levels of gametic phase disequilibrium by replicating self-fertilizations to produce progeny with the same expected inbreeding level. We did not, however, screen all of the allozymes that have been used in other studies of bivalves and, in light of our results, limitation in the number and genomic distribution of markers is a significant constraint to understanding how selection operates. Nevertheless, we conclude that fixation and segregation of allozymes are substantially distorted by strong selection in inbred oyster families. Mapping of QTL for growth, growth physiology, and survival in F_2 hybrid and backcross generations resulting from crosses among these inbred lines are presently underway to test alternative hypotheses for heterosis (HEDGECOCK *et al.* 1996). The association of allozymes and QTL for these traits is of great interest.

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Appendix

Genotypes for 99 progeny and inferred genotypes for hermaphroditic founders of seven inbred families of Pacific oysters:

			Locus													
Family	Individuals	Sex[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
89-1	0	M/F	AD	AA	AA	AC	DD	AA	AA	AB	AB	AF	AC	AA	AB	AC
	1	M	AD	AA	AA	AC	DD	..	AA	AA	AB	AF	AC	AA	..	AC
	2	M	AA	AA	AA	AC	DD	..	AA	AB	AA	AF	AC	AA	..	AC
	3	M	AD	AA	AA	AC	DD	..	AA	AB	AB	AF	AC	AA	..	AC
	4	F	AD	AA	AA	AA	DD	..	AA	AB	AB	AF	AC	AA	..	CC
	5	F	AD	AA	AA	CC	DD	..	AA	AA	AB	AF	AC	AA	..	AC
	6	F	DD	AA	AA	CC	DD	..	AA	AB	AB	AF	AC	AA	..	CC
	7	M	AD	AA	AA	AC	DD	AA	AA	AB	AB	AF	AC	AA	AB	AC
	8	—	AA	AA	AA	AC	DD	AA	AA	BB	AB	AF	AC	AA	BB	AC
	9	—	DD	AA	AA	CC	DD	AA	AA	AA	AA	AF	AC	AA	AB	AC
	10	M	AD	AA	AA	AC	DD	AA	AA	AB	AB	FF	AC	AA	AB	AC
	11	F	AD	AA	..	AC	DD	AA	AA	AB	AB	FF	AC	AA	AB	AC
	12	F	AA	AA	AA	AC	DD	AA	AA	BB	AB	AF	AC	AA	AB	AC
	13	F	AA	..	..	AC	..	AA	..	..	AB	AF	CC	AA	AB	..
	14	F	..	..	..	AC	..	AA	..	..	AB	AF	AC	AA	BB	..
	15	F	..	..	..	AC	..	AA	..	..	AA	AF	AC	AA	AB	..
	16	M	cA	..	..	bC	..	AB	..	..	AB	bF	AA	AA	AB	AA
	17	M	AD	..	..	AA	..	BB	..	..	AA	Ad	AA	AB	..	ee
	18	M	AA	..	..	AC	..	BB	..	..	AA	Ad	AA	AC	AB	eA
89-3	0	M/F	DD	AB	AA	AD	AA	AB	AA	BB	AB	AA	AA	AE	AB	AA
	1	F	AD	Ac	AC	cc	AD	AB	AA	AA	AA	AA	AE	AA	AA	..
	2	F	DD	AB	AA	AD	AA	AB	AA	BB	AB	AA	AA	AE	AB	..
	3	F	DD	AB	AA	AA	AA	AB	AA	BB	AB	AA	AA	AE	AB	AA
	4	F	DD	AA	AA	AD	AA	BB	AA	BB	BB	AA	AA	AA	AB	AA
	5	F	DD	AB	AA	AA	AA	AA	AA	BB	AA	AA	AA	EE	BB	AA
	6	F	DD	AB	AA	AD	AA	BB	AA	BB	AB	AA	AA	EE	AB	AA
	7	F	DD	AA	..	AD	AA	AB	AA	BB	BB	AA	AA	AA	AB	AA
	8	F	DD	AB	AA	AD	AA	BB	AA	BB	BB	AA	AA	AE	AB	AA
	9	F	DD	AB	AA	AD	AA	AB	AA	BB	AB	AA	AA	AE	BB	AA
	10	F	DD	AA	AA	AD	AA	AB	AA	BB	BB	AA	AA	AA	BB	AA
	11	F	DD	AB	AA	AD	AA	AA	AA	BB	AB	AA	AA	AE	AB	AA
	12	—	DD	AB	AA	AD	AA	AB	AA	BB	AB	AA	AA	AE	BB	AA
89-4	0	M/F	AD	AC	AB	AA	AD	AA	AE	AA	AA	AF	AC	AB	AB	AE
	1	M	AA	AA	AA	AA	AD	AA	AA	AA	AA	AA	AA	AA	AA	AA
	2	M	AA	AA	AB	AA	AA	AA	AA	AA	AA	AF	AC	AA	AB	AA
	3	M	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AB	EE
	4	F	AA	AC	AB	AA	AD	AA	AA	AA	AA	AA	AA	AA	AB	AA
	5	F	AA	AC	AA	AA	AD	AA	AA	AA	AA	AF	AA	AA	AB	AA
	6	F	AD	AC	AA	AA	AD	AA	AE	AA	AA	AF	AA	AA	AB	AA
	7	M	AD	AC	AB	AA	AD	AA	AE	AA	AA	AA	AA	AA	AB	EE
	8	F	AA	AA	AA	AA	AD	AA	AA	AA	AA	AA	AA	AA	AA	AA
	9	—	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
	10	—	AA	AA	AB	AA	DD	AA	AA	AA	AA	AA	AC	AB	AB	AA
89-5	0	M/F	AD	AC	AB	AC	AD	AB	AE	AB	AA	AF	AC	AB	BB	AC
	1	F	AA	CC	AB	AA	..	AA	AA	AA	AA	FF	CC	AA	BB	AA
	2	F	AD	AC	AA	AA	AD	AA	AE	AA	AA	AF	CC	AB	BB	AA
	3	M	AD	AC	AB	AA	AD	AB	EE	AA	AA	AF	AA	AB	BB	AA
	4	F	AA	AC	AA	AC	AD	AB	AE	AB	AA	FF	AC	AA	BB	AC
	5	—	AD	AC	AA	AA	AA	AA	AA	AA	AA	AF	AC	AB	BB	AA
92-89-5	6	M	AD	AA	AB	AA	DD	AA	EE	AA	AA	AF	AA	AB	BB	AC
	7	M	AD	AA	AA	AC	DD	AB	AE	AA	AA	AF	AC	AB	BB	AA
	8	M	AA	AA	AA	AC	AD	AA	EE	AB	AA	FF	AA	AB	BB	AC
	9	—	AA	AA	AB	AC	..	..	AE	AB	..	AF	AA	AB	..	..
	10	—	AD	AA	..	AC	..	..	EE	AA	..	FF	AA	AB	..	..

Appendix

Continued

Family	Individuals	Sex[a]	Locus 1	2	3	4	5	6	7	8	9	10	11	12	13	14
	11	—	AD	AC	AA	AA	..	..	EE	AA	..	AF	AA	AA	..	..
	12	—	AA	AA	AA	AC	..	..	AE	AA	..	AF	AC	AB	..	..
	13	—	AD	AA	AA	AC	..	..	EE	AB	..	AF	AA	AB	..	..
	14	—	AA	AA	AB	AA	..	..	AE	AA	..	AF	AC	AA	..	..
	15	—	AA	AC	AB	AC	..	..	AE	AA	..	AF	AC	AB	..	..
	16	—	AA	AC	AB	AA	..	..	AE	AA	..	FF	AA	AA	..	..
	17	—	AA	AC	AA	AC	..	..	EE	AA	..	AF	AA	AA	..	..
	18	—	AA	AA	..	AA	..	..	EE	AA	..	FF	AA	AB	..	..
	19	—	AA	AA	AB	AC	..	..	AE	AB	..	AF	AC	AB	..	..
	20	—	AA	AA	AA	AC	..	..	AE	AA	..	FF	AC	AB	..	..
	21	—	AA	AA	AB	AC	..	..	AE	AB	..	FF	AC	AB	..	..
	22	—	AD	AA	AB	AC	..	..	EE	AA	..	AF	AA	AA	..	..
	23	—	AD	AA	AB	AA	..	..	AE	AA	..	FF	AA	AA	..	..
	24	—	AA	AA	AB	AA	..	..	EE	AB	..	AF	AA	AA	..	..
	25	M	AD	AA	AB	AA	AA	..	AE	AA	AA	AF	AC	AB	..	..
	26	F	AD	CC	AB	AC	AA	..	EE	AA	AA	AF	AC	AA	..	..
	27	F	AA	AA	AB	AA	AD	..	AE	AA	AA	AF	AC	AB	..	..
	28	F	AD	AA	AA	AC	AD	..	EE	AA	AA	AF	AA	AB	..	..
	29	—	AD	AC	AA	AA	DD	..	EE	AA	AA	AF	AA	AA	..	..
	30	—	AD	AA	AA	AC	DD	..	AE	AA	AA	FF	AC	AB	..	..
	31	—	AA	AA	AA	AC	DD	..	EE	AA	AA	AF	AA	AB	..	..
	32	—	AA	AA	AA	AC	DD	..	EE	AA	AA	FF	AA	AB	..	..
	33	M	..	..	..	AC	..	AB	..	..	..	AF	AA	AB	BB	..
	34	M	..	..	..	AC	..	AB	..	..	..	AF	AA	AB	BB	..
	35	M	..	..	..	AA	..	AA	..	..	..	AF	AA	AB	BB	..
	36	M	..	..	..	AA	..	AA	..	..	..	AF	AC	AB	BB	..
	37	M	..	..	..	AC	..	AB	..	..	..	AF	AC	AB	BB	..
	38	M	..	..	..	AA	..	AA	..	..	..	AF	AC	AB	BB	..
89-6	0	M/F	CD	AA	AA	AA	AA	AA	AE	AA	AB	AA	AA	AA	AB	EE
	1	M	CD	AA	AA	AA	AA	AA	AE	AA	AB	AA	AA	AA	AB	EE
	2	F	CD	AA	AA	AA	AA	AA	AE	AA	AB	AA	AA	AA	AB	EE
	3	F	CD	AA	AA	AA	AA	AA	AE	AA	AB	AA	AA	AA	AB	EE
	4	F	CD	AA	AA	AA	AA	AA	AE	AA	AB	AA	AA	AA	AB	EE
	5	M	CD	AA	..	AA	AA	AA	AE	AA	AB	AA	AA	AA	..	EE
	6	F	CD	AA	AA	AA	AA	AA	AE	AA	AB	AA	AA	AA	AB	EE
	7	—	DD	AA	AA	AA	AA	AA	AA	AA	AB	AA	AA	AA	AB	EE
	8	—	CD	AA	AA	AA	AA	AA	AE	AA	AA	AA	AA	AA	AA	EE
89-7	0	M/F	AA	AC	AA	AA	AD	AA	AA	AB	AA	AA	AA	AI	AB	AB
	1	M	AA	AA	AA	AA	AD	AA	AA	AA	AA	AA	AA	AA	BB	AA
	2	F	AA	AC	AA	AA	AD	AA	AA	AA	AA	AA	AA	AI	BB	AB
	3	F	AA	AC	AA	AA	AA	AA	AA	AB	AA	AA	AA	AI	AB	AB
	4	F	AD	AA	AA	AA	AA	AA	AE	AA	AA	FF	AC	Ab	BB	AA
	5	M	AA	AC	AA	AA	DD	AA	AA	AA	AA	AA	AA	AI	AB	AA
	6	M	AA	AC	AA	..	DD	AA	AA	..	AA	..	AA	..	..	..
	7	F	AA	AC	AA	AA	DD	AA	AA	BB	AA	AA	AA	AI	BB	AA
	8	F	AA	AA	AA	..	AA	AA	AA	AB	AA	AA	AA	AI	..	AA
92-1	0	M/F	AA	AC	AB	AA	AA	..	..	AA	AB	AF	..	DH	..	..
	1	—	AA	AC	BB	AA	AA	..	..	AA	AB	AF	..	DH	..	..
	2	—	AA	AC	AB	AA	AA	..	..	AA	BB	AA	..	DH	..	..
	3	—	AA	CC	BB	AA	AA	..	..	AA	AB	AF	..	DD	..	..
	4	—	AA	AC	BB	AA	AA	..	..	AA	BB	AA	..	DH	..	..
	5	—	AA	AA	AB	AA	AA	..	..	AA	AB	AF	..	DH	..	..

[a] Loci numbered in order: (1) *Aat,* (2) *Acon-1,* (3) *Acon-2,* (4) *Adk,* (5) *Dia,* (6) *G-3-pdh,* (7) *Gpi,* (8) *Dap-2,* (9) *Idh-2,* (10) *Lap-2,* (11) *6-pgdh,* (12) *Pgm,* (13) *Mp-1* and (14) *Sdh;* . ., unscored genotypes; individuals numbered 0 are hermaphrodite founders.