Context-Dependent Survival Differences Among Electrophoretic Genotypes in Natural Populations of the Marine Bivalve *Spisula ovalis*

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ABSTRACT

We investigated the relationships between allozyme genotypes at nine polymorphic loci and survival in a natural population of the bivalve *Spisula ovalis* sampled on three occasions (1993, 1994, and 1995) in three different sites (2855 individuals analyzed). This species displays annual growth lines allowing identification of annual cohorts. Therefore we could avoid cohort mixing, a frequent bias in such studies, and evaluate the consistency of the observed effects across cohorts and sites. Significant viability differences were observed both among alleles and between heterozygotes and homozygotes at some loci. Multiple-locus heterozygosity was positively correlated with viability in the 1993–1994 period, but not in the 1994–1995 interval. The observed selective effects were significantly dependent on the cohort and the site considered. A bibliographic survey suggests that such variability is a common feature of studies analyzing heterozygosity-survival relationships. Two explanations are consistent with our results. First, allozyme genotypes may have direct effects on viability that interact with subtle environmental variation in a complex and unpredictable way. Second, allozyme genotypes may be transiently associated with other viability genes responsible for heterotic effects. In any case, the results militate against allozyme loci being themselves consistently overdominant for viability in natural populations.

NEUTRALITY is a desired quality for any genetic marker to be used in population studies. Since the discovery of electrophoretic variation (LEWONTIN and HUBBY 1966), molecular markers have been largely used in population genetics and their neutrality has been the subject of intense debates. The most widely used molecular markers are allozymes, although an increasing proportion of studies use DNA markers (AVISE 1994; MITTON 1994). Unlike many anonymous DNA sequences, allozymes have known physiological functions and therefore are or have been a target for natural selection, as evidenced by adaptations and structure-function relationships observed at the protein level (reviewed in GILLESPIE 1991). However, the question of how many allozyme polymorphisms are maintained by some form of natural selection and how many are neutral and transient remains under discussion (LEWONTIN 1991). Predictions of the neutral theory concerning broad statistical properties such as the distribution of the genetic diversity H_e have generally been confirmed (see for a recent example WOODWARK *et al.* 1993), although the tests may suffer from lack of statistical power, especially to detect some selective regimes that mimic neutral distributions (GILLESPIE 1991). However, many case studies document selective effects on allozymes, following two approaches. The first one consists in a detailed functional dissection of allozyme properties relevant to fitness (*e.g.,* WATT 1983, review in GILLESPIE 1991). The second one, which will be the focus of the present study, is a correlative approach, *i.e.,* the analysis of relationships between fitness components and genotypic variation at allozyme loci. One of the main components of fitness is viability, and viability-genotype relationships have been investigated in various organisms (plants: SCHAAL and LEVIN 1976; amphibians: SAMOLLOW 1980; Drosophila: MUKAI *et al.* 1974; marine bivalves: ZOUROS *et al.* 1983; DIEHL and KOEHN 1985). Relationships between enzyme heterozygosity and viability have received much attention, as heterozygote superiority is the simplest way to maintain polymorphism under selection.

Evidence for heterozygosity-survival relationships in natural populations have been accumulating rather slowly, although a publication bias may favor significant results (BOOTH *et al.* 1990; GAFFNEY 1990). A high proportion of these studies have concerned marine bivalves, and have yielded inconsistent results. Several cases of superior viability of heterozygotes have been found (KOEHN *et al.* 1973; ZOUROS *et al.* 1980; DIEHL and KOEHN 1985). However some nonsignificant results (GAFFNEY 1990) and even significantly lower survival of the heterozygote (ALVAREZ *et al.* 1989) have also been reported. Although this inconsistency may partly rely on the heterogeneity of the sampling designs and statistical methods used, it is also possible that no general trend actually exists in these organisms. Importantly, the repeatability in time and space of the presumed selective effects has not been statistically assessed in the studies

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cited above. An interesting attempt is GAFFNEY's (1990) study on *Mytilus edulis,* in which the positive heterozygosity-viability relationship initially observed failed to repeat; however no statistical test was provided on whether genotype-specific survival significantly differed across samples.

We here present a study of genotype-viability relationships in a marine bivalve, *Spisula ovalis* (Sowerby), which was designed to meet four desirable criteria for such studies: (i) the individuals used were recruited and spent their whole life in their native wild population; (ii) a large number of individuals (2855) and polymorphic loci (9) were analyzed; (iii) age was determined using a character independent of size (annual growth lines), allowing discrimination of the effects of differential survival *vs.* differential growth; and (iv) comparisons were made between temporal samples of the same cohorts, not between different cohorts, allowing discrimination of intrageneration effects due to differential viability from among-generation differences of unknown origin. Specifically, we asked the following questions: (i) Do different alleles at a given locus confer different individual survival abilities? (ii) Does heterozygosity influence survival rates? and (iii) Are the observed survival differences repeatable from year to year, and across independent generations and sites?

MATERIAL AND METHODS

Sampling: The surf clam *S. ovalis* (Bivalvia: Mactridae) is an infaunal suspension-feeder occupying sandy bottoms up to 50 m deep along the European Atlantic coasts. The planktonic larval life lasts up to several weeks, while the adult lifespan can reach 10 years. Recruitment period varies with temperature (and latitude) and is mainly restricted to the summer (between July and October) in the locality sampled for the present study. Individuals were dredged at three sites (A, B, and C) in the Glénans Archipelago, off the Bay of Concarneau (Brittany, France) (47°25'N, 4°00'W). A size bias may be present, as small individuals may be resuspended more easily than large ones during dredge movements. However there is no evidence for size differences among electrophoretic genotypes within cohorts (P. DAVID, unpublished results), so that sampling bias cannot result in apparent survival differences between genotypes. The same three sites were resampled on three occasions in 1993 (April 1), 1994 (May 5), and 1995 (May 3). In 1995, the population of site C apparently went extinct, as no living individual of Spisula was caught despite repeated attempts. Spisula shells display narrow, conspicuous shell lines related to winter growth pauses, which have proven to be reliable indicators of age (see DAVID *et al.* 1995). On this basis, we distinguished annual cohorts, *i.e.,* groups of individuals born on the same year, within each sample. Ten different cohorts were represented in the cumulated sample of 1993, 1994, and 1995 (cohorts 1985 to 1994). However, some cohorts were absent some years at some sites, and the frequency of the 1985 and 1986 cohorts remains very low in all samples. Sample sizes for each year, cohort, and site are given in Table 1. Sites A, B, and C are separated by only a few hundred meters (with no obvious physical or ecological barrier among them) and share similar ecological conditions, *i.e.,* subtidal sandflats, with no seagrass, sheltered from oceanic swell by the numerous surrounding rocks and islets.

TABLE 1

Sample size of *Spisula ovalis* per sampling year, site, and cohort

Year	1993			1994			1995		
Site	A	B	C	A	B	C	A	B	C
Cohort									
1985	0	3	0	0	0	0	0	0	0
1986	11	14	19	1	0	0	0	0	0
1987	80	100	64	46	50	17	60	64	0
1988	39	54	36	142	71	50	90	56	0
1989	119	24	32	133	75	58	84	58	0
1990	76	4	15	167	33	16	181	46	0
1991	3	0	0	70	5	5	124	37	0
1992	0	0	0	25	25	3	108	54	0
1993	0	0	0	0	0	0	111	42	0
1994	0	0	0	0	0	0	15	39	0

Given the duration of the larval pelagic phase and the potential for dispersion in Spisula, these sites must be considered as "open populations" (ROUGHGARDEN *et al.* 1985), *i.e.,* there is not necessarily genetic continuity among successive cohorts at a given site. For this reason, each group of individuals of the same cohort from the same site must be considered as a separate genetic entity in our samples. As a convention, these entities are referred to as "populations" in the following.

Individual genotypes at nine polymorphic enzymatic loci (*PGI, PGM, LAP, AAT, IDH, EST, GAL, PGD,* and *LAP2*) were determined using horizontal starch-gel electrophoresis. Detailed procedures are given in DAVID *et al.* (1995) for the first seven loci. *PGD* and *LAP2* were run on Tris-citrate pH 8.0 gels (*cf.* DAVID *et al.* 1995) using muscle and digestive gland extracts, respectively, and were stained according to PASTEUR *et al.* (1987). The genetic structure of this sample (Hardy-Weinberg equilibrium, differentiation among sites and cohorts) has been presented elsewhere (P. DAVID, M.-A. PERDIEU, A.-F. PERNOT and P. JARNE, unpublished results). The overall result was that significant heterozygote deficiencies were detected every year within all sites and cohorts, and there were significant genetic differences in both space (among sites) and time (among cohorts).

Data analysis: We first tested whether allelic frequencies remained identical over time. Given the genetic stucture mentioned above, pooling of individuals over cohorts or sites would yield spurious results as the within-generation temporal variation would be confounded with the spatio-temporal genetic structure. Therefore we compared the allelic contingency tables at each locus for the three (or two) temporal resamplings of each cohort in each site. This was done using an approximate exact test for population differentiation (RAYMOND and ROUSSET 1995a) performed with the software GENEPOP 2.0 (RAYMOND and ROUSSET 1995b). In this test, the probability of occurrence of the observed allele × population contingency table under the null hypothesis of no differentiation is compared to that of samples with random reallocation of alleles across populations, generated using a Markov chain process. The null hypothesis is rejected when the sum of the probabilities of the observed sample and less probable ones is, say, less than 0.05. To obtain standard errors one order of magnitude lower than the estimated probabilities, we set the numbers of steps of the Markov chain to 4×10^6 for all tests. Exact tests were performed when the sample size exceeded 30 for at least two recaptures of a given cohort in a given site, in order to exclude tests with low power. Exact tests are of

interest as they are not sensitive to low-frequency classes and therefore allele pooling is not required. However they do not allow a global approach to the selective effects over all populations, nor do they provide a test of the homogeneity of these effects across sites, cohorts, or years. Therefore we also tested the stability of allele frequencies using a Poisson log-linear model (MANLY 1985, p. 46–51), after pooling alleles of nearest electrophoretic mobilities until all allelic classes had frequencies higher than 0.05. The model can be summarized as follows:

$$\text{Log}\ (G_{ijk}) = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + \delta_i a + \eta_{ik} a + \epsilon_{ijk}, \quad (1)$$

where G_{ijk} is the number of genes observed in the allelic class i in year j in population k, μ is the mean logarithm of G_{ijk}, α_i is the main effect of allelic class i, β_j is the main effect of the year of sampling j (three modalities, 1993–1995), γ_k is the main effect of population k, $(\beta\gamma)_{jk}$ and $(\alpha\gamma)_{ik}$ are the two-way interaction terms, a is the age in years (quantitative variable), δ_i is the main effect of age in allelic class i, η_{ik} is the effect of age in allelic class i in population k, and ϵ_{ijk} is the error term. G_{ijk} has a Poisson distribution and was treated accordingly (Log transformation and Poisson errors, see CRAWLEY 1993, p. 226). Model 1 can be interpreted as follows. The first five terms permit the total number of genes of each allelic class (summed over all populations and years), and the total number of genes in any given population in any given year, to be set to the observed values. The $(\alpha\gamma)_{ik}$ interaction fits the variation in the relative proportions of allelic classes across populations. The $\delta_i a$ term fits the variation of these proportions in time (*i.e.*, the selective effects on allele frequencies). The $\eta_{ik} a$ term accounts for heterogeneous selective effects across populations.

In order to analyze the relative viabilities of heterozygotes versus homozygotes at each locus, we fitted the following generalized linear model:

$$\text{Log}\left(\frac{H_{kl}}{1 - H_{kl}}\right) = \alpha_k + \beta a + \gamma_k a + \epsilon_{kl}, \quad (2)$$

where H_{kl} is the heterozygosity at the locus considered for individual l in population k (0 if the individual is homozygous at this locus, 1 if heterozygous), α_k is the effect of population k, β is the main effect of age a, γ_k is the effect of age in population k, and ϵ_{kl} is the error term. H_{kl} is a binary variable and was treated accordingly, using logit transformation and Bernoulli error (CRAWLEY 1993, p. 291).

Multilocus heterozygosity (*MLH*) was computed for all individuals by summing up H values over loci ($0 \leq MLH \leq 8$; no individual had $MLH = 9$). The resulting variable has neither a normal nor a binomial error, and is therefore impractical to handle as the dependent variable in a model similar to (2). Therefore, instead of testing directly the effect of age on *MLH* as in (2), we tested the null hypothesis of temporal stability of the relative proportions of *MLH* classes, after pooling the extreme classes to obtain reasonable sample sizes (classes $MLH = 0$ and 1 pooled together; same for $MLH = 6$, 7, and 8; total: six classes). This test is based on a Poisson log-linear model (MANLY 1985, p. 46–51) in which the dependent variable is N_{ijk}, the number of individuals in the *MLH* class i in year j in population k. In contrast with *MLH*, the distribution of N_{ijk} is simple (Poisson distribution). The model is

$$\text{Log}\ (N_{ijk}) = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + \delta_i a + \eta_{ik} a + \epsilon_{ijk}, \quad (3)$$

where all terms have the same meaning as in model 1, except that N_{ijk} is a number of individuals (not genes) and the subscript i refers to *MLH* class i (not to the allelic class i). Note that the *MLH* class is treated as a factor (six *unranked* modalities) and not as a quantitative variable. Absolute mortality rates, *i.e.*, λ_i values in the equation

$$N_i(t) = k_i e^{-\lambda_i t}$$

(where k_i is a constant, and $N_i(t)$ is the number of individuals in the *MLH* class i at time t) cannot be estimated from our dataset, as the sampling effort is not standardized across years (MANLY 1985). However model 3 allows estimation of relative survival rates of different *MLH* classes. The class $MLH \geq 6$ was arbitrarily chosen as the reference class and assigned a value of $\lambda_6 = 0$. The estimated values of $(\delta_6 - \delta_i)$ in model 3 give the class-specific mortality rates (λ_i values) relative to the reference class.

In model 3, the null hypothesis of stability of the *MLH* distribution is tested against no specified alternative. However, we were especially interested in testing this null hypothesis against the hypothesis of a linear (either positive or negative) effect of *MLH* on survival, as is usually tested on other fitness traits such as growth (*e.g.*, DAVID *et al.* 1995). This was done using the following model:

$$\text{Log}(N_{ijk}) = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + \partial ai + \eta_k ai + \epsilon_{ijk}, \quad (4)$$

where the first six terms are as in (3). The relative survival of *MLH* class i (noted δ_i in model 3) is now assumed to be a linear function of i with slope δ. Therefore the effect of age on N_{ijk} is expressed as the product δai. This term represents all changes of the *MLH* distributions in time that can be explained by a linear effect of individual *MLH* on survival. The $\eta_k ai$ term describes the variation of this effect across populations (indexed by k). Note that, as for model 3, no assumption on the distribution of *MLH* is needed in model 4.

Models 1 to 4 allow tests for effects of genotype on survival constant over time (referred to as *linear selection* in the following), as a is a numerical quantitative variable. Temporally variable effects were fitted simply through replacing a by Y, the *sampling year* factor in all models. When Y is used, the heterogeneity between years is tested, regardless of the existence of a consistent direction in selective effects in 1993–1994 and 1994–1995.

Hypothesis testing for models 1 to 4 was done through model simplification procedures. The significance of a term of the model is evaluated by a chi-square test on the change in residual deviance when this particular term is removed from the model (CRAWLEY 1993, p. 188). Terms are removed sequentially, starting from the complete model and removing first the highest-level interactions. The significance of temporal variation in selective effects was assessed by a chi-square test on the difference in deviance between the model using the Y factor and the same model using the variable a. This can be done as the latter model is included in the model with Y, meaning that replacing Y by a is indeed a model simplification. All computations for models 1 to 4 were done using the software GLIM 4.0 (BAKER 1987). Samples of a given population in a given year were excluded from the log-linear Poisson models when their size was less than 15 individuals or when the same population had not been resampled in another year (final sample size: 2546 individuals). All individuals were included in the single-locus heterozygosity analysis (model 2), except when the sample size was lower than five individuals over the 3 years.

Our sampling scheme sometimes involved a large number

TABLE 2

Probabilities of type I error given by the exact tests for population differentiation among successive resamplings of the same populations (cohort × site)

Cohort	1987	1988	1989	1990	1991	1987	1988	1989	1990	1988	1989	Global test	
Site	A	A	A	A	A	B	B	B	B	C	C	Chi-square (22 d.f.)	*P*
Loci													
PGI	0.363	0.965	0.835	0.883	0.832	0.084	0.419	0.719	0.073	0.196	0.911	19.1	0.639
PGM	0.728	**0.039**	0.872	0.559	0.248	0.849	0.896	0.665	0.296	0.379	0.595	18.1	0.699
LAP	0.430	0.064	**0.047**	0.519	0.350	0.233	0.346	0.195	**0.045**	0.056	**0.023**	44.5	**0.003**
AAT	0.648	0.393	0.714	0.056	0.631	0.625	**0.033**	0.068	0.295	0.933	0.442	27.5	0.194
IDH	0.087	0.416	0.257	0.070	0.808	0.686	0.185	0.426	0.335	0.857	0.513	24.8	0.308
EST	0.802	**0.042**	0.505	0.165	0.924	0.933	1.000	0.701	0.067	0.183	**0.038**	28.1	0.173
GAL	0.405	0.066	0.599	0.492	**0.009**	0.366	0.709	0.340	0.356	0.304	0.364	30.5	0.107
PGD	0.730	0.926	0.177	0.149	0.246	**0.031**	0.785	0.491	0.766	**0.003**	0.982	32.1	0.077
LAP2	0.334	0.442	0.666	0.300	0.533	0.889	0.551	0.266	0.722	0.869	0.263	16.0	0.817
Global test Chi-square (18 d.f.)	15.2	29.2	16.8	25.1	20.3	1.92	16.6	18.8	27.2	29.3	23.0	All tests Chi-square (198 d.f.) = 240.6	
P	0.65	**0.046**	0.535	0.123	0.315	0.38	0.548	0.407	0.076	**0.045**	0.191	*P* = **0.021**	

Chi square values and their associated probabilities are global tests using Fisher's method. *P* values are across loci (last row), populations (last column) or both (All tests, bottom right). Boldface denotes significance at the 0.05 level.

of replicated independent tests. In order to obtain global significance values, we used Fisher's method for combining independent test results (SOKAL and ROHLF 1995).

RESULTS

Variation of allele frequencies among years within populations: The results of exact tests are presented in Table 2. Fisher's test on the whole table is significant ($P = 0.021$), indicating some temporal changes in allele frequencies within populations. Significant tests are rather evenly distributed across populations and loci, although a larger number of significant tests is detected at the *LAP* locus than at the other loci. The results of the log-linear Poisson model 1 are summarized in Table 3. This model confirms the existence of significant differences in allele frequencies between populations. Significant variation among successive resamplings of the same populations is also observed. The $\delta_i a$ term in (1), and the corresponding terms in (2) to (4), represent the amount of selective effects that are uniform across populations and are referred to as the *uniform components* in the tables. For model 1, the uniform component is never significant, *i.e.*, there are no general trends across all populations as far as allele-specific viabilities are concerned. In contrast, overall highly significant effects appear when variation of the selective effects across populations is taken into account ($\eta_{ik} a$ term). The results obtained when the variable a is replaced by the factor Y in model 1 are qualitatively similar to those of model 1 (not shown). For all loci, the extra deviance explained when Y is used (instead of a) is not significant (last column in Table 3), indicating a lack of significant temporal variation in viability differences among alleles.

Variation of heterozygosity among years within populations: The results of the single-locus heterozygosity analysis (model 2) are given in Table 4. Overall, there are significantly different levels of single-locus heterozygosity among populations and among successive resamplings of the same populations. Heterozygosity within populations increased between 1993 and 1995 for six loci out of nine, as attested by positive coefficients for the uniform component ($\beta > 0$). Moreover, all cases of significant variation in heterozygosity (*EST*, *GAL* and, to a lesser extent, *PGI*) are associated with positive coefficients. However, the uniform component itself is significant for *EST* only, and the overall test is not significant. In contrast, most significant effects are found in the population-specific component, indicating that the selective effects observed may not be generalized across populations. No cases of temporal variation were observed in these selective effects.

The distribution of multiple-locus heterozygosity did not vary across populations (Table 5). Model 3 pointed to significant changes of *MLH* distributions over years within populations. In contrast to the single-locus analysis, the uniform component was significant, whereas population-specific effects were absent. Model 4 showed that these types of variation could only be partly explained by a linear effect of *MLH* on survival, as the uniform component in model 4 was only marginally significant ($P < 0.10$, two-tailed test). However, there was significant temporal variation in this effect. We therefore analyzed the 1993–1994 and 1994–1995 periods separately using model 4. A significant effect of *MLH* on survival [δai term in (4)] was observed in the 1993–1994 period, when high *MLH* classes achieved

TABLE 3

Intrageneration variation in allele frequencies within populations of *Spisula ovalis*

				Linear selection						Temporal variation	
		Population effect[b]		All selective effects[c]		Uniform component[d]		Population-specific component[e]		All selective effects[f]	
Locus	*Nc*[a]	DD	*P*	DD	*P*	DD	*P*	DD	*P*	DD	*P*
PGI	2	27.06 (14)	**0.019**	27.49 (15)	**0.025**	0.02 (1)	0.890	27.47 (14)	**0.017**	2.10 (9)	989
PGM	2	28.44 (14)	**0.012**	12.09 (15)	0.672	0.50 (1)	0.480	11.60 (14)	0.639	3.15 (9)	0.958
LAP	4	57.69 (42)	0.054	80.76 (45)	**0.0008**	1.19 (3)	0.755	79.58 (42)	**0.0004**	28.67 (27)	0.377
AAT	2	18.48 (14)	0.186	15.35 (15)	0.427	0.08 (1)	0.783	15.27 (14)	0.360	16.34 (9)	0.060
IDH	2	6.33 (14)	0.957	12.36 (15)	0.652	0.81 (1)	0.369	11.55 (14)	0.643	9.07 (9)	0.431
EST	2	25.34 (14)	**0.031**	14.83 (15)	0.464	0.90 (1)	0.342	13.93 (14)	0.455	11.44 (9)	0.247
GAL	5	94.65 (56)	**0.0009**	75.53 (60)	0.085	1.71 (4)	0.789	73.82 (56)	0.055	42.83 (36)	0.201
PGD	4	32.84 (42)	0.844	59.03 (45)	0.078	2.58 (3)	0.460	56.45 (42)	0.067	30.82 (27)	0.279
LAP2	4	40.40 (42)	0.541	43.94 (45)	0.516	1.94 (3)	0.584	42.00 (42)	0.471	24.79 (27)	0.586
Global test			**0.0001**		**0.0042**		0.9322		**0.0011**		0.393

Values are deviances (DD) explained by several components of model 1, with corresponding d.f. in parentheses, and and probabilities of type I error (*P*) given by chi-square tests. Boldface denotes significance at the 0.05 level.

[a] Number of allelic classes after allele pooling.

[b] Among-population differences in allele frequencies [term $(\alpha\gamma)_{ik}$ in model 1].

[c] Terms $\delta_i a + \eta_{ik} a$.

[d] Term $\delta_i a$ only.

[e] Term $\eta_{ik} a$ only.

[f] Differences in deviance between model 1 and the same model when a is replaced by Y.

better survival than low ones (Figure 1). In 1994–1995, no general trend was apparent, and the δai term was not significant. Note that the relationship between *MLH* and mortality in 1994–1995 is similar to that in 1993–1994 (Figure 1), with the exception of the *MLH* $\geq$ 6 class, which apparently suffered an unexpected high mortality in 1994–1995. However, this could be due to sampling variance.

DISCUSSION

The main result of this study is to show the existence of viability differences both at the allelic and genotypic

TABLE 4

Results of linear models using single-locus heterozygosities (model 2)

		Linear selection				Temporal variation
Locus	H_{obs}[a]	Population effect[b]	All selective effects[c]	Uniform component[d]	Population-specific component[e]	All selective effects[f]
PGI	0.121	**0.020**	0.058	0.009 (0.999)	**0.041**	0.983
PGM	0.210	**0.042**	0.552	0.017 (0.752)	0.488	0.227
LAP	0.521	**0.049**	0.274	0.059 (0.317)	0.269	0.219
AAT	0.337	0.570	0.222	0.063 (0.317)	0.216	0.575
IDH	0.464	0.688	0.177	−0.097 (0.094)	0.248	0.968
EST	0.523	0.908	**0.004**	0.119 (**0.038**)	**0.009**	0.208
GAL	0.611	0.116	**0.012**	0.097 (0.100)	**0.018**	0.076
PGD	0.401	0.207	0.908	−0.043 (0.480)	0.896	0.086
LAP2	0.600	0.912	0.899	−0.032 (0.584)	0.877	0.575
Global test		**0.039**	**0.006**	(0.170)	**0.011**	0.253

Boldface denotes significance at the 0.05 level.

[a] Observed heterozygosities.

[b] *P* values for among-population differences in heterozygosity (term α_k in model 2).

[c] *P* values for terms βa and $\gamma_k a$ together.

[d] Term βa only, estimates of the age effect (β) with *P* values in parentheses.

[e] *P* values for term $\gamma_k a$ only.

[f] *P* values for the differences in deviance between model (2) and the same model when a is replaced by Y.

TABLE 5

Intrageneration variation in the distribution of *MLH*

Model	Population effect[a]		Linear selection						Temporal variation	
			All selective effects[b]		Uniform component[c]		Population-specific component[d]		Uniform component[e]	
	DD	*P*	DD	*P*	DD	*P*	DD	*P*	DD	*P*
3	71.78 (70)	0.419	74.13 (75)	0.507	13.72 (5)	**0.017**	60.41 (70)	0.786	6.43 (5)	0.267
4	71.78 (70)	0.419	14.55 (15)	0.484	2.72 (1)	0.099	11.83 (14)	0.620	4.64 (1)	**0.031**
4, 1993–1994 only	61.53 (70)	0.755	14.83 (13)	0.318	6.33 (1)	**0.012**	8.50 (12)	0.745	—	—
4, 1994–1995 only	71.78 (70)	0.419	10.09 (11)	0.522	0.62 (1)	0.433	9.48 (12)	0.488	—	—

Values are deviances (DD) explained by several components of models 3 and 4, with corresponding d.f. in parentheses, and probabilities of type I error (*P*) given by chi-square tests.

[a] Among-population differences in *MLH* distribution [term $(\alpha\gamma)_{ik}$ in models 3 and 4].

[b] Terms $\delta_i a + \eta_{ik} a$ for model 3 and $\delta ai + \eta_k ai$ for model 4.

[c] Term $\delta_i a$ for model 3 and δai for model 4.

[d] Term $\eta_{ik} a$ for model 3 and $\eta_k ai$ for model 4.

[e] Differences in deviance between model 3 or 4 and the same models where *Y* is substituted for *a*. The three-way interactions (population-specific components) are never significant in (3) or (4) and were therefore not taken into account to estimate temporal variation in selective effects. —, no temporal variation can be assessed when two sampling dates only are taken into account.

levels in the populations of *S. ovalis* studied. Differences among genotypes mainly consist of instances of heterozygote advantage, at the single- and multiple-locus levels. The detailed study of linear models and exact tests highlighted considerable variability of selective effects among populations (*i.e.*, cohorts and sites). Indeed allele frequency changes occurred at different loci, depending on the population (Table 2). Moreover most significant changes in allele frequencies and single-locus heterozygosity values are concentrated in the interactions with the population factor, while the uniform components are not significant (Tables 3–5). Therefore no consistent survival advantage or handicap is associated with any genotype or allele in our dataset. Such inconsistency had already been evaluated qualitatively by GAFFNEY (1990), who noticed the lack of repeatability of age-heterozygosity associations in the mussel *M. edulis*, over three independent experiments. Among-population variation in selective effects may be explained by two features of the Spisula populations studied: (i) each population may have experienced a unique environmental history, if environmental conditions vary in space (among sites) and/or in time (among cohorts); (ii) each population may have a unique genetic background, as different populations may come from different parental stocks. Indeed, the spatio-temporal genetic structures observed in Spisula (P. DAVID, M.-A. PERDIEU, A.-F. PERNOT and P. JARNE, unpublished results) and other bivalves (HEDGECOCK 1994) are consistent with each cohort in a given site descending from a limited number of parents, and relatively low effective population sizes, although larval dispersion has been classically claimed to promote large effective sizes and genetic mixing.

A significant positive effect of *MLH* on survival is observed between 1993 and 1994. Although globally consistent across populations, this effect was due to different loci in different populations. This effect was not observed in 1994–1995 and model 4 points to a significant difference in selective regimes between the two periods. This can be explained by either a change in environmental selective pressures or by the exhaustion of the genetic variance under selection. However, selection on single locus heterozygosity is maintained over both periods (temporal variation not significant).

In contrast with heterozygosity-growth or heterozygosity-size relationships (reviewed in MITTON and GRANT 1984), studies of relationships between allozyme heterozygosity and survival are relatively scarce in marine bivalves, as well as in other organisms. A literature survey of these studies is summarized in Table 6. We focused on the sampling design and statistical methods used, bearing in mind the desired criteria for survival analysis mentioned in the Introduction. Most authors concluded that heterozygosity and survival were either unrelated or positively related. The same study sometimes produced both results when analysing different samples or periods. On the whole, we can hardly conclude that there is definitive evidence for generalized genotype-dependent survival, for two main reasons. First, a diversity of statistical methods is used, which makes between-study comparisons or generalizations difficult. For example, the same data on toad tadpoles gave no significant heterozygosity-survival relationship using contingency chi-square tests (SAMOLLOW 1980) and a significant positive relationship when reanalyzed using sign tests (SAMOLLOW and SOULE 1983). The statistical analyses may be individual, *MLH* class or pop-

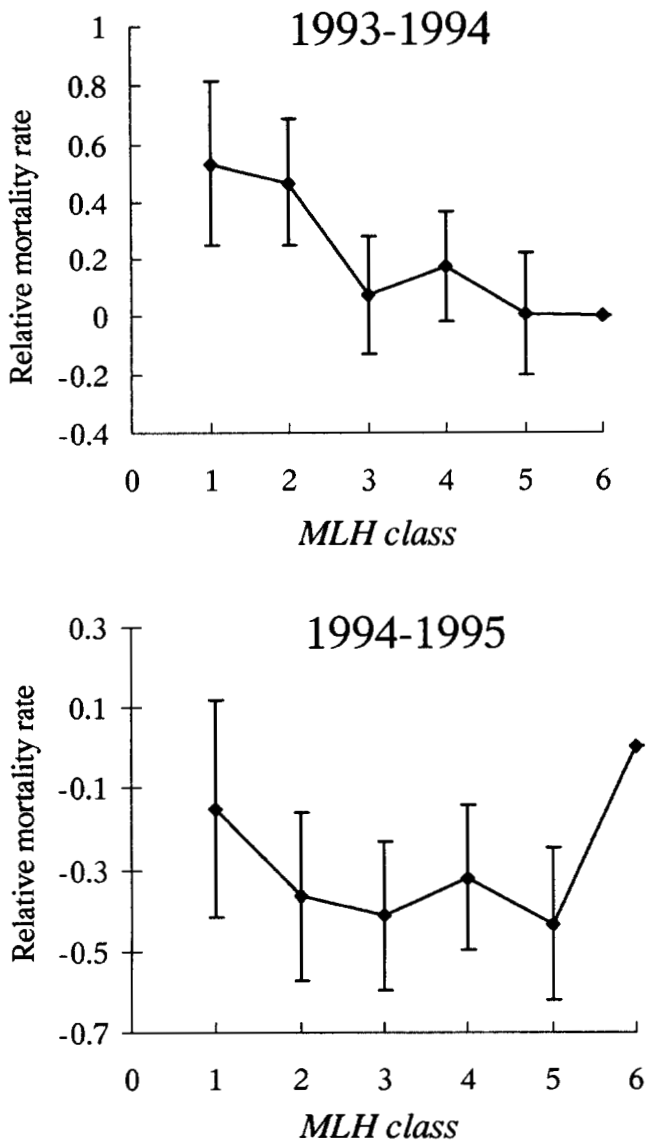

FIGURE 1.—Relative mortality rates λ_i for *MLH* classes i = 1 to 6 (see text for details on how classes were constituted). λ_6 was arbitrarily set to 0, and other λ_i given are relative to λ_6. Data are displayed as estimated $\lambda_i \pm$ SD. Estimates of the linear effect of *MLH* on survival (δ values in model 4) are 0.1032 ± 0.0411 and -0.0292 ± 0.03725 for 1993–1994 and 1994–1995, respectively.

ulation-based. Population or class descriptors such as heterozygote deficiency or excesses (WATT 1977; SAMOLLOW 1980) or class viability estimates (ZOUROS *et al.* 1983; DIEHL and KOEHN 1985; ALVAREZ *et al.* 1989) are used in unweighted linear models despite nonnormal distributions and heterogeneous variances. Second, with the exception of SAMOLLOW (1980), none of these studies meets all four criteria described in the Introduction. Most studies have large sample sizes (of the order 10^3) but it is generally impossible to exclude biases due to population or cohort mixing. As individuals cannot be unambiguously aged in most species, cohorts cannot be followed through successive samplings. An exception is the study of GREEN *et al.* (1983) on *Macoma balthica*, where annual shell lines allow age determination as in Spisula. However there is only one sampling date in this study, and the sample size is small (154 individuals). Cohort mixing may be an important bias as substantial genetic differentiation between cohorts has been observed in various species including molluscs (*e.g.*, JOHNSON and BLACK 1984; P. DAVID, M.-A. PERDIEU, A.-F. PERNOT and P. JARNE, unpublished results) and fishes (JORDE and RYMAN 1996). Whatever the origin of this differentiation, it may obscure intrageneration viability effects if different cohorts are compared. Therefore the results of studies in which several cohorts are mixed in the analysis should be interpreted with caution.

In contrast with previous studies, the present study allows direct evaluation of differences in heterozygosity within a given site and a given cohort, resampled at different occasions, and therefore quantification and testing of relative viabilities of genotypes in natural populations, using the appropriate log-linear model (MANLY 1985). The significant variability in selective effects and overall weak, though significant, heterozygote advantage observed here are consistent with many null results previously obtained in marine bivalves (see Table 6), frequently in papers reporting heterozygote advantage for other samples or periods (ZOUROS *et al.* 1983; DIEHL and KOEHN 1985; GAFFNEY 1990; BORSA *et al.* 1992). Moreover, the only study that meets the four criteria stated in the Introduction (SAMOLLOW 1980) gave results qualitatively similar to the present study, with significant selective effects highly fluctuating in time in an unpredictable way and overall very slight evidence for heterozygote advantage. We conclude that both the present study and the literature survey provide limited evidence for positive associations between heterozygosity and survival and that these associations consistently exhibit great variability, both in time and across populations. Temporal fluctuations in apparent selection coefficients on allozyme genotypes have also been described at the intergeneration level in a long-term study of Daphnia wild populations (LYNCH 1987).

Hypotheses on the origin of genotype-dependent survival mainly fall into two categories, depending on whether or not the marker genes themselves are assumed to determine viability differences. This is an important issue, intimately related to the problem of the neutrality of allozyme markers (LEWONTIN 1991). If we assume that the marker genotypes directly influence viability, we then have to deal with how the genetic polymorphism is maintained in the long term. Two chief types of selective regime may play this role. The first is overdominance, where the heterozygous genotypes consistently enjoy a net selective advantage. The second is genotype-environment interaction, meaning that different genotypes are favoured in different environmental conditions, whether in time or in space (cf. *e.g.*, GILLESPIE and TURELLI 1989). Overdominance is not supported by the present dataset, given the high

TABLE 6

Summary of a bibliographic survey on the relationships between allozyme heterozygosity and survival

Reference	Conclusions[a]	Species	No. of individuals[b]	Origin[c]	Conditions[d]	Mixing[e]	Test[f]	Description of principal tests
Marine bivalves								
ALVAREZ *et al.* (1989)	0/−	*Ostrea edulis*	641 (5)	Lab	CC	Y	LR (CL)	Unweighted correlation *MLH*/viability estimates of *MLH* classes (4 points)
BORSA *et al.* (1992)	0	*Ruditapes decussatus*	55 (7)	NP	CC	Y	LR (IND)	Correlation *MLH*/time to death
	+	*R. decussatus*	220 (7)	NP	NP	N	M (IND)	*t*-test on mean *MLH* between two sampling dates
DIEHL and KOEHN (1985)	0/+	*M. edulis*	1555 (5)	NP	SCC	Y?	M (IND), DIST (CL)	*t*-test on mean *MLH* between resamplings, G-test on *MLH* distributions
FUJIO *et al.* (1979)	0/+	*Crassostrea gigas*	1310 (2)	NP	NP	N	DIST (CL)	Tests of heterozygote excess compared to Hardy-Weinberg, interpreted as evidence for selection
GAFFNEY (1990)	0	*M. edulis*	738 (5)	NP	SCC	Y	—	No test; qualitative comparison between *SLH* of two recaptures
GREEN *et al.* (1983)	+	*M. balthica*	154 (6)	NP	NP	N	LR (IND)	Correlation *MLH*/individual age
KOEHN *et al.* (1973)	+	*Modiolus demissus*	2539 (1)	NP	NP	N (S)	—	No test; graph of heterozygote deficiency *vs.* estimated age (size class)
ZOUROS *et al.* (1983)	0/+	*C. virginica*	3169 (3–4)	NP	SCC	N	DIST (CL)	G test on *MLH* distributions between samples of different age
Others								
MITTON and KOEHN (1975)	+	*Fundulus heteroclitus*[g]	1362 (12)	NP	NP	Y? (S)	M (IND)	*t*-test on mean *MLH* of successive resamplings
MUKAI *et al.* (1974)	0	*Drosophila melanogaster*	10560 (7)	Lab	CC	Y	M (IND)	Comparisons of survival rates of offspring from controlled crosses (among *SLH* or *MLH* classes)
SAMOLLOW (1980)	0	*Bufo boreas*[h]	3708 (9)	NP	NP	Y	LR (GR)	Regression of heterozygote deficiency or excess of successive resamplings on sampling date
SAMOLLOW and SOULE (1983)	0/+	Subsample of the above	1332 (9)	NP	NP	Y	M (LOC, GR)	Sign test on heterozygosity variation between two resamplings, across loci and populations
SCHAAL and LEVIN (1976)	+	*Liatris cyclindracea*[i]	1848 (5)	NP	NP	N	LR (IND)	Correlation between *MLH* and estimated individual age
WATT (1977)	+	*Colias philodice*[j]	324 (1)	NP	NP	N	LR (GR)	Unweighted correlation between heterozygote excess and sampling date (4 dates)
	+	*C. philodice*	235 (1)	NP	NP	N	LR (GR)	Rank correlation between mean estimated age and heterozygote excess (6 population)

[a] Authors' conclusions on the results of the study. Positive, negative, and nonsignificant heterozygosity-survival relationships are noted +, −, or 0, respectively. When several samples gave different results, both are mentioned.

[b] Number of individuals analyzed with the number of allozyme loci analyzed in parentheses.

[c] Origin of collected individuals: NP, natural populations; Lab, laboratory strains.

[d] Conditions of maintenance of individuals during the survival study; NP, natural population; CC, controlled conditions; SCC, semi-controlled or not fully natural conditions.

[e] Can population or cohort mixing be excluded as a potential source of observed effects? Y, yes; N, no; Y?, yes with restrictions (imprecise delimitation of cohorts); (S), individual age determined from size classes and therefore possible confusion between growth and survival effects.

[f] Type of statistical test used: LR, linear relationship; M, comparison among means; DIST, comparison among distributions. The statistical unit considered in the tests (= one data point) is given in parentheses: IND, individual; CL, *MLH* or genotypic class; GR, group of individuals (population or sample); LOC, locus; *SLH*, single-locus heterozygosity; —, no test.

Species as follows: [g]fish; [h]toad; [i]herb; [j]butterfly.

between-population differences. The relationship between *MLH* and survival observed is consistent across populations, but does not involve the same loci, as expected under overdominance. Genotype-environment interactions could produce the among-population variation observed in this study. However, (i) this hypothesis does not explain why *MLH* should be related to survival within populations, in a given period, as we found in 1993–1994 and (ii) the three sites are very close to each other and ecologically similar. Of course we cannot exclude the possibility of subtle environmental differences between sites. In the absence of a detailed study of environmental parameters and their influence on physiological allozyme functions, the genotype-environment interaction hypothesis is *ad hoc,* and could fit any kind of results. Such a detailed study may be found in WATT (1983) and WATT *et al.* (1983), where ecological, genetical, and biochemical data suggest that the polymorphism at the *PGI* locus in Colias butterflies is partly maintained by a combination of overdominance and selection in heterogeneous environments characterized by different thermal regimes. An alternative approach consists in comparing the results obtained from allozymes and presumably neutral DNA markers (POGSON and ZOUROS 1994). Their results suggest that only allozyme genotypes correlate with fitness traits (in their case, body size). However the difference between the two classes of genetic markers were only marginally significant, so this experiment needs to be replicated.

Alternatively, viability differences among allozyme genotypes may reflect their association with viability genes (the *associative hypothesis*). This is consistent with the observed variability of selective effects among populations, as interlocus associations may be transient and highly dependent on the genetic background of the population. The positive relationship observed between *MLH* and survival (or any fitness trait) is expected if allozyme heterozygosity is correlated with that of other genes, either because of transient linkage desequilibria due to low effective population size or because of identity disequilibria generated by partial inbreeding (HOULE 1994). Indeed, allozyme-associated heterosis has been observed for the two measurable fitness traits in Spisula, *i.e.,* growth (DAVID *et al.* 1995) and survival (present study). The associative hypothesis implies that populations are not at Hardy-Weinberg equilibrium, because of either small effective size or inbreeding. Spisula populations indeed display significant heterozygote deficiencies (P. DAVID, M.-A. PERDIEU, A.-F. PERNOT and P. JARNE, unpublished results), like most marine bivalves (ZOUROS and FOLTZ 1984). However these deficiencies generally cannot be attributed to inbreeding, although very low levels of inbreeding cannot be excluded (GAFFNEY 1990; P. DAVID, M.-A. PERDIEU, A.-F. PERNOT and P. JARNE, unpublished results). The inbreeding hypothesis thus has weak empirical support. Moreover, inbreeding can generate apparent heterozygote advantage at marker loci, but cannot generate the apparent advantages for particular alleles observed here (Tables 2 and 3). As previously mentioned a given cohort in a given site may be engendered by a limited number of parents. Heterogeneity among parental stocks and drift effects could result in transient interlocus associations, as well as in different genetic backgrounds in different cohorts.

In conclusion, this study, and other ones, show context-dependent survival differences between allozyme genotypes. This leaves two possible explanations, *i.e.,* (i) that each genotype directly influences viability, although in a environment-dependent manner, and (ii) that observed viability differences rely on transient associations between electrophoretic genotypes and viability genes, therefore being sensitive to variation in both the environmental conditions and the genetic background. Hypothesis (ii) is consistent with previous knowledge on the population structure and heterosis in *S. ovalis.* Although we have mainly focused on viability, other fitness-related traits such as growth have yielded similar results (MITTON and GRANT 1984) and the same hypotheses have been put forward to explain them. Following POGSON and ZOUROS (1994), an interesting approach would be to assess apparent fitness effects of other markers, such as microsatellites, for which neutrality in the short term (within one generation) is less questionable than for allozymes.

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