The Effect of Glucose-6-Phosphate Isomerase Genotype on in Vitro Specific Activity and in Vivo Flux in Mytilus edulis

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Four samples of the mussel Mytilus edulis were taken between 1984 and 1987 from Stony Brook, New York, and used to study the glucose-6-phosphate isomerase (GPI) polymorphism in this species. In vitro specific activity and in vivo flux measured in the same animals were found to be significantly correlated. A significant effect of GPI genotype on flux was observed in one of the samples; overall, significant evidence of effect of genotype on enzyme activity was also obtained. GPI activities of common genotypes tend to deviate less from the population mean than those of rare (frequency less than 5%) genotypes. This suggests the possibility that rare GPI genotypes are rare as a consequence of having biochemical properties that deviate from an

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optimum level and, therefore, having a lower fitness. In support of this hypothesis, we found in one of our samples that shell length is a concave function of GPI activity with an intermediate optimum activity level.

KEY WORDS: glucose-6-phosphate isomerase; in vitro specific activity; in vivo flux; Mytilus edulis; selection for biochemical intermediacy.

INTRODUCTION

It is well known that genetic variants of enzymes often, perhaps commonly, exhibit differences from one another in biochemical properties (e.g., specific activity, $V_{\text{max}}$, $V_{\text{max}}/K_m$, thermostability). Whether or not such biochemical variability deriving from genetic polymorphism has experimentally measurable and biologically significant effects on metabolism and fitness is still poorly understood. Koehn et al. (1983) describe several examples in which enzyme activity has significant effects on metabolism and fitness. On the other hand, Dean et al. (1986) show that for enzymes with low control coefficients, rather large variations in enzyme activity can result in much smaller changes in metabolic flux and fitness.

Glucose-6-phosphate isomerase (GPI; EC 5.3.1.9) catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate in glycolysis and gluconeogenesis. The mass action ratios (i.e., [F6P/G6P]) are near the equilibrium constant ($K_{eq} = 0.3$) in virtually every studied species. In spite of serving at the branch point of glycolysis and the pentose shunt, GPI is thought to provide primarily a coupling function between this branch point and phosphofructokinase/fructose-1,6-biphosphatase, without any regulatory function (Newsholme and Start, 1973; Atkinson, 1977; Cameselle et al., 1980; Ebberink and de Zwaan, 1980). Differences in catalytic properties associated with the GPI enzyme polymorphism might therefore not seem as likely to produce variations in glycolytic flux as differences in some other enzymes.

There is, nevertheless, evidence that catalytically differentiated GPI allozymes can influence glycolytic rate. The studies of GPI in Colias butterflies (cf. Watt and Boggs, 1987) by Watt and his collaborators have demonstrated the manner by which genotype-dependent catalysis can influence glycolytic flux and thereby various energy-dependent measures of performance such as flight capacity.

The frequencies of GPI alleles are spatially clinal and correlated with water temperature in a wide diversity of organisms distributed along the U.S. Atlantic coast, including the bivalve Mytilus edulis (Koehn et al., 1976), the eel Anguilla rostrata (Koehn and Williams, 1978), the teleost fishes Menidia beryllina (Johnson, 1974) and Fundulus heteroclitus (Powers and Place,
1978), the sand crab *Emerita talpoida* (Corbin, 1977), and the anemone *Metridium senile* (Hoffman, 1981b, 1985). These similar patterns of geographic differentiation led Hoffman (1981a) to search for a temperature-dependent catalytic basis for GPI clines in *Metridium senile* and Hall (1985) to do the same in *Mytilus edulis*; in both cases allelic differences in $V_{\text{max}}/K_m$ were observed, consistent with the latitudinal frequency differences.

However, Hall (1985) was concerned with only 2 common electrophoretic alleles, of the more than 30 known to be segregating in natural populations of *Mytilus edulis* from the Western Atlantic. Earlier studies on the population genetics of this species (cf. Koehn et al., 1976, 1984) pooled less common alleles in order to give several "synthetic" alleles that could be more easily treated statistically.

In this paper, we summarize the results of four studies of GPI variation in *Mytilus edulis*. We focus on several issues that until now have received little attention. First, we address the effect of genotype on enzyme activity using *all* the GPI genotypes observed in our samples. Second, using a modification of the tritiated glucose method of Zaba and Davies (1980), we compare the rate of *in vivo* flux from glucose-6-phosphate to fructose-6-phosphate with *in vitro* specific activity. Third, we study an association between the mean GPI activity of each genotype and the frequency of that genotype. We suggest that genotype frequency in populations is a reflection of the activity of the GPI allozymes and provide some evidence that this activity may influence a surrogate measure of fitness.

**MATERIALS AND METHODS**

In Long Island (New York) Sound the mussel *Mytilus edulis* usually settles in June and July. Spat settles where populations of this species are already established, thereby bringing young animals to multiage structured populations. Spat also settle on a raft that is placed in the Stony Brook harbor (Long Island, New York) each spring and removed from the water in the winter, when it is cleared of all animals and plants living on it. Each year, large samples are routinely taken from this raft prior to its removal, transferred to mesh bags, and returned to the sound at Stony Brook for growth until later sampling. All the samples used in this study were taken from one of these two settlements, which provide the unique opportunity to study natural populations, our ultimate research subject, as well as to sample from adjacent, single-year cohorts, for more controlled studies.

*Sample 1.* Mussels of the 1983 cohort (i.e., settled in summer, 1983) were sampled in April 1984. Animals were brought to the laboratory and held in recirculating seawater (15–20°C, 25% S) without food prior to analysis.
Crude homogenates of digestive gland were prepared in pH 8.7 buffer (0.5%, w/v) containing 75.0 mM Tris/HCl, 2.0 mM Na₂EDTA, 0.7 mM phenylmethylsulfonylfluoride, and 0.2% β-mercaptoethanol. Digestive gland tissue was solubilized by sonication in the cold and centrifuged at 10,000g for 1 hr. Supernatant was used as the crude enzyme preparation.

GPI activity was measured in duplicate for 338 individuals in the direction of gluconeogenesis at 20°C. The assay mixture contained 0.10 M NaMOPS (constant ionic strength), 1.0 mM NADP, 8.0 mM MgCl₂, 2.5 mM fructose-6-phosphate, 1.25 units glucose-6-phosphate dehydrogenase, pH 7.1, in a final volume of 0.5 ml (Hall, 1985). The reaction was initiated by the addition of 20.0 μl crude enzyme homogenate and was monitored by following the reduction of NADP at 340 nm on a Gilford 2400-2 UV/VIS recording spectrophotometer interfaced with a Technical Systems Consultants 6800 microcomputer.

Total soluble protein was measured in duplicate according to Bradford (1976) using bovine serum albumin as the standard. Enzyme activity was expressed as specific activity [nmol product · min⁻¹ · (mg protein)⁻¹].

For electrophoresis, frozen digestive gland was sonicated in 100 μl of buffer (0.05 M Tris–HCl, 20% glycerol, pH 8.0) and was centrifuged at 10,000g for 10 min. GPI genotypes in the supernatant were determined by horizontal starch gel electrophoresis, according to Koehn et al. (1984).

Sample 2. Mussels were sampled at low tide from a natural population in the Stony Brook harbor, several times between January and April 1985. Sampled animals were therefore a mixed age group, consisting mostly of large adults. Animals were held for 7 to 13 days under the same laboratory conditions as above.

In vivo flux at the metabolic step catalyzed by GPI (i.e., rate of isomerization of glucose-6-phosphate to fructose-6-phosphate) was measured in triplicate in gills as the rate of detrinitiation of [2⁻³H]glucose to water, by a modification of the method of Zaba and Davies (1980) for mantle slices (Winshell and Ertl, 1986). Mantle tissue could not be used in this study because it is susceptible to seasonal gametogenic variation. Additionally, slices of the mantle of a single animal were found to be significantly heterogeneous for GPI activity.

Since gill slices also produced heterogeneous flux measures, whole gills were used (one or two per animal, depending on weight) and preincubated in the mineral salts medium of Zaba and Davies (1980) for 1 hr in a shaking temperature-controlled bath. A mixture of cold and tritiated glucose was then added, to a final concentration of 5 mM glucose and 0.2 μC · (ml medium)⁻¹, at a pH of 7.5. After 2 hr, 0.5-ml aliquots of the reaction mixture were added to an equal volume of 6% perchloric acid to stop the reaction. Tritiated water
was recovered from this mixture by micro vacuum distillation (Winshell and Ertl, 1986). Radioactivity of 0.2-ml aliquots was measured by liquid scintillation in 5.0 ml of Scintiverse II. Counts were corrected for background and quenching, and results are expressed as $\mu$mol $^3$H$_2$O $(g\ wet\ tissue)^{-1} \cdot (2\ hr)^{-1}$. Detritiation was linear over time for 4 hr. There was no significant effect of sampling date or duration of acclimation, as determined by ANOVA.

Flux was measured at two different temperatures, 175 individuals at 15°C and 181 at 29°C. Temperatures were selected to represent both normal and stress conditions in nature. At 15°C, mussels are eurythermal, but 29°C produces such metabolic stress that animals can survive only short exposures to this temperature. Nonetheless, animals are known to be exposed to that temperature during summer low tides in the Stony Brook harbor.

A small fragment of mussel, including mantle, digestive gland, and adductor muscle, was sonicated for genotype determination by the same method as above.

**Sample 3.** Mixed-age adult animals were collected from a natural population from the Stony Brook harbor as before, between January and March 1986. This third study was designed specifically to address the question of the relationship between GPI in vitro specific activity and GPI in vivo flux. One gill was therefore used to determine specific activity and the other to determine flux, right and left gills being haphazardly chosen from each of 356 animals for each measurement. Specific activity was determined as before, except that the gill was ground in homogenization buffer set at pH 7.1 (the same as the assay mixture). Flux was measured as above, but only in duplicate and at 29°C. Again, no effects due to sampling date or acclimation period were detected. Genotypes were determined as above.

**Sample 4.** The fourth and final sample was taken to provide an estimate of the variability among years, by replicating the study on a single year cohort (sample 1) as closely as possible. Animals of the 1986 cohort were sampled in April 1987. Genotypes and specific activity were measured using the digestive gland of 251 animals, as in that study (except that the pH of the homogenization buffer was again 7.1).

Table I summarizes our samples, measures of GPI performance, and assay conditions.

All chemicals were from Sigma, except fructose-6-phosphate (Boehringer), [2-$^3$H]glucose (Amersham), and Scintiverse (Fisher). Statistical analyses were done on an IBM PC/XT or on an IBM 4361, using the BIOMETRY package of computer programs (Sokal and Rohlf, 1981) and statistical procedures of SAS (Freund and Littell, 1981).
Table I. Sampling Scheme for GPI Activity Measurements in *Mytilus edulis*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sampling dates</th>
<th>Age structure</th>
<th>Measure of GPI activity</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Apr. 1984</td>
<td>Single-year cohort</td>
<td>Specific activity</td>
<td>20</td>
</tr>
<tr>
<td>2a</td>
<td>Jan.–Apr. 1985</td>
<td>Mixed ages</td>
<td>Flux</td>
<td>15</td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td></td>
<td>Flux</td>
<td>29</td>
</tr>
<tr>
<td>3a</td>
<td>Jan.–Mar. 1986</td>
<td>Mixed ages</td>
<td>Specific activity</td>
<td>20</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td></td>
<td>Flux</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>Apr. 1987</td>
<td>Single-year cohort</td>
<td>Specific activity</td>
<td>20</td>
</tr>
</tbody>
</table>

RESULTS

Specific Activity and Flux

Since these studies used two different measures of GPI activity, namely, *in vitro* specific activity and *in vivo* flux, it is appropriate to consider whether these two measures estimate the same property of the reaction catalyzed by GPI.

Rate of release of tritium to water is an imperfect measure of metabolic flux at the GPI step, since there is also partial transfer of $^3$H between the C2 of glucose-6-phosphate and the C1 of fructose-6-phosphate (Katz and Rognstad, 1976; Rose, 1981), resulting in detritiation in the tricarboxylic acid cycle. Since glucose-6-phosphate is also a source of the pentose shunt, detritiation may also occur in this pathway, but its flux in *Mytilus* is much smaller than that of glycolysis. The exchange/transfer ratio for GPI in the forward direction has been reported as 1 for some systems (Rose, 1981). Even though it can differ in other organisms and experimental conditions, as well as in the reverse direction, due in particular to isotope discrimination (Rose and O'Connell, 1961), we estimate that about 50% of the detritiation measured is due to flux at the GPI step. This clearly indicates that we must expect considerable noise in the measurement of *in vivo* flux.

The appropriate data set to test the relationship between specific activity and flux is from sample 3, in which specific activity and flux were measured in equivalent parts of the same individual animals. The two measures of enzyme activity are significantly correlated (Fig. 1), both for the measurements on individuals ($r = 0.23$, df = 354, $P < 0.0001$) and when these are grouped by genotype ($r = 0.38$, df = 32, $P < 0.03$). The lower significance of the result based on the means is clearly due to the reduced number of degrees of freedom, since the proportion of the variance of one variable explained by the other is even greater in this case than for the individual measurements.

The relatively low correlation between specific activity and flux was expected, for the reasons considered above. Nevertheless, the significance
level of the correlation between both measures among individual animals assures that specific activity and flux are determined by a common set of biological parameters and can both be used as measures of GPI enzyme performance. It also demonstrates that detritiation is a useful, if imperfect, measure of flux at the GPI step. We will use *activity* to denote reaction performance of the GPI enzyme, independently of how it was determined, and *specific activity* when it is necessary to refer specifically to the *in vitro* measure.

**Effect of Genotype**

The fact that specific activity and flux show a significant correlation when grouped by genotype may suggest a genotypic effect on activity. The
appropriate test of this hypothesis is an ANOVA of activity by genotype. Since a standard ANOVA requires normally distributed data, and both activity and flux of sample 3 were not, the Box-Cox transformation (e.g., Sokal and Rohlf, 1981, p. 423) was applied to both variables. ANOVAs of the normalized data were not significant (Table II).

The other samples can also be used to address this question, since each provides a measure of GPL activity and genotype. The results from all the studies are in Table II. Some of the samples yield significant results, while others do not. On the other hand, all six $P$ values are smaller than 0.5, a very unlikely event under the assumption of no effect of genotype. A two-way ANOVA on the overall data, with study as one factor and genotype as the other, would have had greater statistical power than individual ANOVAs, but the different variances among studies violate one of the basic assumptions of ANOVA. Simply standardizing to a common variance is inappropriate, since activity may also be correlated with size (see below); standardization would be complex and probably unsatisfactory. Moreover, there are rank-order genotypic differences among some studies, which would further complicate a two-way analysis of variance.

However, since each row in Table II represents an independent study, with the single exception of 3a and 3b, significance values can be combined by Fisher’s method (Fisher, 1944, p. 99) in an overall test of the null hypothesis of no genotypic effects. Because of the independence requirement of this test, either 3a or 3b must be dropped. To be conservative we drop 3b (the one closest to significance). Fisher’s method, an early tool of what has recently

<table>
<thead>
<tr>
<th>Sample</th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>2a</td>
<td>19,155</td>
<td>1.78</td>
<td>0.0290*</td>
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<tr>
<td>2b</td>
<td>26,154</td>
<td>1.86</td>
<td>0.0112*</td>
</tr>
<tr>
<td>3a*</td>
<td>33,322</td>
<td>1.02</td>
<td>0.4406</td>
</tr>
<tr>
<td>3b*</td>
<td>33,322</td>
<td>1.08</td>
<td>0.3496b</td>
</tr>
<tr>
<td>4*</td>
<td>28,222</td>
<td>1.52</td>
<td>0.0533</td>
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</table>

Fisher’s combination of $P$ values

<table>
<thead>
<tr>
<th>df</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>27.82</td>
</tr>
</tbody>
</table>

a These samples were nonnormally distributed and were Box-Cox transformed to normality for the ANOVA.

b This value was not included in Fisher’s method for combining significance values (see text).

* Significant at the 5% level.

** Significant at the 0.05% level.
been called meta-analysis (see, e.g., Hedges and Olkin, 1985), requires only statistical independence among individual tests (e.g., Sokal and Rohlf, 1981, p. 779). In particular, it is totally unaffected by possible genotype–environment or tissue–activity interactions.

The result of this test is a highly significant rejection of the null hypothesis (Table II). GPI genotype has a significant effect on enzyme activity, measured either as specific activity or as flux, but we are not able to detect this effect in every single study.

**Genotypic Frequency and Enzyme Activity**

As shown in Fig. 2, the mean activities of the common genotypes tend to deviate less from the grand mean than the mean of the rare ones. While rare genotypes can have any level of activity, common genotypes—those with a frequency of, say, 5% or more—have more intermediate levels of activity. This pattern is of course expected on purely statistical terms, since means of large samples have smaller standard errors than means of small samples from the same population. To test whether the common genotypes are closer to the sample mean in our data, above and beyond this statistical tendency, we computed for each genotype the statistic $|z_i|$ (Zar, 1974):

$$|z_i| = |(\bar{x}_i - \bar{x})/(\sqrt{s^2/n_i})|$$

where $\bar{x}_i$ is the mean of genotype $i$, $\bar{x}$ the grand mean of the whole sample, $n_i$ the genotypic sample size, and $s^2$ the overall sample variance. Since we were unable to detect heteroscedasticity among genotypes within each study, the overall sample variance is a more efficient estimator of the true variance than the variances associated with each genotype, because these are necessarily based on smaller samples. By dividing the deviation between each genotypic mean and the grand mean by the standard error of that genotypic mean, we effectively remove the statistical artifact we want to avoid.

Whether a particular genotype is higher or lower than the mean is not the point here but, rather, whether common genotypes tend to cluster closer to the overall mean than would be expected by chance alone. Hence, we take the absolute value of the standardized mean.

Clearly, if the pattern shown by our data is only a statistical artifact, there should be no relationship between $|z|$ and genotypic frequency, as measured by a regression of the former on the latter. This was verified empirically by performing 100 randomizations of the individual observed GPI specific activity data from the 1983 cohort, randomly sorted into simulated "genotypic" classes of the observed frequencies. From this simulation we obtained one regression coefficient different from zero at the 0.01 level, five at
Fig. 2. Relationship between GPI in vitro specific activity and GPI in vivo flux of each genotype and frequency of that genotype in four samples of Mytilus edulis. Units as in Fig. 1.
the 0.05 level, and eight at the 0.10 level, in excellent agreement with the expectation.

If, however, the observed pattern is real with a probable biological cause, the precise relationship to be expected between $|z|$ and $n$ is less clear. A possible biological cause of the pattern shown by the data is that in this species, rare GPI genotypes are rare as a consequence of having lower fitness, as a result of biochemical properties which deviate from an optimal level, as per Latter (1975).

In this case, $|z|$ should be large for small $n_i$ and small for large $n$, that is, the relationship between $|z|$ and $n$ should be negative. As shown in Table III, the slope of the regression of $|z|$ on sample size is negative in all six studies but statistically significant in none of them. The fact that all the slopes are negative as expected is by itself significant evidence against the null hypothesis, but the point can be further tested by a complete analysis of covariance (Table III). The slopes of all six studies are not significantly heterogeneous, and the combined slope is significant at the 0.02 level.

We conclude that, even though we are unable to show it in any isolated sample, the mean activities of rare genotypes do indeed tend to deviate more from the sample average than the mean activity of common genotypes. This result suggests that there is selection for biochemical intermediacy of the GPI enzymes of *Mytilus edulis*.

This selection is not necessarily due to catalytic differences among GPI allozymes, in terms of $k_{cat}$ or $K_m$. Genotypic differences in enzyme activity can also be due to enzyme concentration, which in turn can depend on very tightly linked regulatory sequences, transcriptional and translational efficiency, stability of the mRNA and of the allozymes themselves, etc. The molecular causes of the activity differences observed in this study, interesting in their own right, cannot at present be ascertained and would require a different

<table>
<thead>
<tr>
<th>Sample</th>
<th>$b$</th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
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<tbody>
<tr>
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<td>0.1164</td>
</tr>
<tr>
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<td>-0.0127</td>
<td>1.18</td>
<td>0.48</td>
<td>0.4961</td>
</tr>
<tr>
<td>2b</td>
<td>-0.0210</td>
<td>1.25</td>
<td>1.35</td>
<td>0.2563</td>
</tr>
<tr>
<td>3a</td>
<td>-0.0088</td>
<td>1.32</td>
<td>2.62</td>
<td>0.1154</td>
</tr>
<tr>
<td>3b</td>
<td>-0.0011</td>
<td>1.32</td>
<td>0.04</td>
<td>0.8423</td>
</tr>
<tr>
<td>4</td>
<td>-0.0057</td>
<td>1.27</td>
<td>0.16</td>
<td>0.6912</td>
</tr>
<tr>
<td>Homogeneity of slopes</td>
<td>5,156</td>
<td>0.40</td>
<td>0.8480</td>
<td></td>
</tr>
<tr>
<td>Common slope</td>
<td>-0.0086</td>
<td>1,166</td>
<td>5.52</td>
<td>0.0200*</td>
</tr>
</tbody>
</table>

*Significant at the 5% level.
experimental approach (e.g., Aquadro et al., 1986). Nevertheless, these specific molecular mechanisms are irrelevant for our finding of a relationship between enzyme activity and genotypic frequency and the possibility of selection against deviant genotypes. Natural selection results in differential mortality regardless of the causes, catalytic or otherwise, of the phenotypic variation present in the population.

Activity and Fitness

In mussels, size at a specific age is one indicative measure of fitness. Indeed, in indeterminately growing poikilotherms, size is correlated with several fitness components such as reproductive effort, survivorship, and so forth (Peters, 1983). An analysis of the association between genotype and size can therefore provide a test of the effects of genotype on a surrogate measure of fitness. From the preceding section, we expect this relationship as one of stabilizing selection, since uncommon genotypes exhibit a more deviant phenotype than expected by chance. If indeed rare, phenotypically deviant genotypes are rare because they have lower fitness, we expect a curvilinear relationship between size and GPI activity, such that both low and high activities are associated with small animals, and intermediate activity is associated with largest animals. This can be tested directly in our single cohort samples (samples 1 and 4).

The deviation of GPI activity of rare genotypes from the population mean was most apparent in sample 1. Hence, it was in this sample that we had expected to see the strongest relationship between size and activity. This was not the case—this sample showed no relationship between individual size and activity. Sample 4, on the other hand, did show the predicted relationship between GPI activity and size (Fig. 3). The curve shown is the best polynomial fit to the data ($F = 24.98$, $P < 0.0001$, $r^2 = 0.17$; this is a significantly better fit to the data than a linear model, but a cubic equation does not fit significantly better).

Since the results were different in the two samples, they do not provide clear evidence either for or against our hypothesis of selection for intermediate GPI activity. The only evidence remains the fact that the most deviant genotypes are rare.

DISCUSSION

The fact that the rate of detritiation is an imperfect measure of glycolytic flux through the GPI step militates against detecting a significant association between in vitro specific activity and in vivo flux. Any detected relationship is therefore very conservative, and yet we found the correlation between these
two measures of GPI activity to be very highly significant. When some of the noise is averaged out by correlating the genotypic means, rather than the individual measurements, the proportion of the variation of one measure determined by the variation of the other becomes considerably greater (14% as opposed to 5%).

Kacser and Burns (1981) have shown that in a multienzyme system, the expected relationship of enzyme activity to metabolic flux is nonlinear. The fact that we observed a linear relationship between GPI activity and GPI flux may seem to contradict this result. However, we studied the enzyme activity and flux of the same metabolic step, and not the effect of the activity of one enzyme on the overall flux of the pathway, so our result has no direct bearing on this theoretical analysis.

The same cohort that provided sample 1 has also been studied in the context of the relationship between multiple-locus heterozygosity and growth.
While a significant positive correlation in September 1983 was reported for this cohort (Koehn and Gaffney, 1984), no correlation was found in April 1984 (Diehl and Koehn, 1985), when our sample 1 was taken. Furthermore, the frequencies of the heterozygosity classes in the two samples are different, which suggests that differential mortality may have occurred during the winter.

Most relevant for the present study is the fact that the frequencies of GPI genotypes were found to deviate significantly from Hardy–Weinberg expectations in September 1983, with a marked deficiency of heterozygotes (Koehn and Gaffney, 1984). By April 1984, however, the frequencies of GPI genotypes from this cohort fit the Hardy–Weinberg expectations (this study; Diehl and Koehn, 1985). This is confirmed by a R × C test of the frequencies of GPI homozygotes versus heterozygotes, showing them to be significantly different. The evidence supporting selection at the GPI locus in this cohort prior to the sampling for this study is therefore compelling. As a result of such prior screening by selection, the differences among the surviving genotypes might be small and hard to detect. It is therefore not surprising that in this sample specific activity appears neither different among genotypes nor related to size.

All the animals in sample 4 were collected at the same time from a small area of the raft and were, thereafter, kept in a single mesh bag until brought to the laboratory for study. Hence, they have all experienced the same ecological conditions such as temperature, salinity, etc., throughout their adult lives. We can therefore reject the possibility that the observed relationship between enzyme activity and size (Fig. 3) is just a reflection of broad environmental variation that might affect both GPI activity and size.

This curvilinear relationship implies an intermediate maximum size, which is very different from the monotonically increasing concave fitness function between activity and fitness suggested by Gillespie (1976) and Hartl et al. (1985). A possible explanation for this discrepancy is the fact that the environmental conditions experienced by this cohort varied considerably since its settlement in the summer and the next spring, when the animals were sampled and studied. As noted by Hartl et al. (1985), when the environment varies in such a manner as to alter the activity–fitness function, the overall result of natural selection may be selection for an optimum intermediate enzyme activity.

An important aspect of our results is the general lack of consistency, since our analyses produced different results in different samples. Moreover, when we looked for correlations between mean genotypic activities among studies, we generally did not find them. It is difficult to assess the exact reasons for this inconsistency, since the studies differ in several factors, such as assay temperature and pH, year of sampling, and age structure of the population sampled.
One important uncontrolled factor is the different environments experienced by each sampled population. Genotype x environment interactions could therefore produce different effects of the same genotypes in annually different environments. For instance, in the present studies, even single-year cohorts sampled from the same site at the same time of the year, but in different years (samples 1 and 4), yielded different results. It is quite possible that phenotype (i.e., enzyme activity) is unimportant in relatively benign environments and that the metabolic consequences of phenotypic diversity are expressed only in more stress-producing ecological circumstances. For instance, it has been recently established in *Mytilus edulis* that the effect of enzyme heterozygosity on physiological performance depends on the environment (Koehn and Bayne, 1988), and Samollow and Soulé (1983) have also reported a similar observation in a natural population of the toad *Bufo boreas*. In this respect, it is noteworthy that both of the 1985 studies, involving samples from the same natural population, show significant effects of genotype, while in the 1986 sample neither specific activity nor flux is different among genotypes (Table II).

Probably because of the statistical problems associated with the study of very rare genotypes, their biochemical characteristics have not been adequately investigated, but there are nevertheless some studies. Costa *et al.* (1983) showed that various biochemical properties of an esterase in *Drosophila melanogaster* differed especially between a rare homozygous mutant and two common homozygous variants, and Nigro *et al.* (1985) showed that this rare mutant was strongly selected against when homozygous. These authors postulated that differences in larval yield between adults of rare versus common esterase-6 genotypes were due to differences in catalytic properties of the allozymes in the physiological process of sperm use. Chambers *et al.* (1984) noted that rare variants of the alcohol dehydrogenase locus in *D. melanogaster* could be classified into one of two groups that corresponded to the respective biochemical properties of the two common genotypes. A similar observation was made by Eanes and Hey (1986) for specific activity of rare glucose-6-phosphate-dehydrogenase variants from natural populations of the same species (although some rare variants had severely reduced function). In *Colias* butterflies the most striking pattern is heterozygote superiority, but several examples of common-genotype intermediacy can be clearly seen. In *C. eurysteme* biochemical properties of common phosphoglucose isomerase variants (equivalent to GPI in the present study) were intermediate to rare variants with respect to thermostability and *K_m* for fructose-6-phosphate (Watt, 1977). In *C. philodice eriphyle* the *V_max* and *V_max* ratios of common GPI genotypes were often intermediate to those of rare genotypes (Watt, 1983).

The results of these few studies can be classified into two groups: (1) those that show the biochemical properties of rare variants clustered around
those of the common ones and (2) those in which the common variants are
biochemically intermediate. The present study of the GPI polymorphism in
*Mytilus edulis* falls in the second category and suggests the possibility of
selection against rare, biochemically deviant genotypes. It seems possible that
rare genotypes are maintained by a mutation–selection balance, but further
study, involving in particular controlled varying and/or stressful environ-
ments, would be needed to establish this unequivocally.

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