Expression of Overdominance for Specific Activity at the Phosphoglucomutase-2 Locus in the Pacific Oyster, *Crassostrea gigas*

Grant H. Pogson

Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A9

Manuscript received November 7, 1989
Accepted for publication January 11, 1991

ABSTRACT

Environmental and genetic components of specific activity variation at the phosphoglucomutase-2 locus in the Pacific oyster, *Crassostrea gigas*, were examined to assess the direct role played by this polymorphism in a heterozygosity/growth relationship. Both environmental variables studied, season and intertidal position, exerted highly significant effects on phosphoglucomutase specific activity but no interactions occurred between these factors and *Pgm-2* genotype. Highly significant differences were also detected between *Pgm-2* genotypes. The three most common heterozygotes ($Pgm\text{-}2^{92/100}$, $Pgm\text{-}2^{96/100}$ and $Pgm\text{-}2^{100/104}$) consistently expressed greater specific activities than the $Pgm\text{-}2^{92/92}$, $Pgm\text{-}2^{96/96}$, $Pgm\text{-}2^{100/100}$ and $Pgm\text{-}2^{104/104}$ homozygotes. Overall, the specific activities of heterozygotes for the $Pgm\text{-}2^{100}$ allele exceeded heterozygotes by 24% and 20% in the mantle and adductor muscle tissues, respectively. Heterozygotes formed between the three less frequent $Pgm\text{-}2^{92}$, $Pgm\text{-}2^{96}$ and $Pgm\text{-}2^{104}$ alleles differed sharply from those possessing the $Pgm\text{-}2^{100}$ allele in being indistinguishable from homozygotes. The possibility of these patterns arising from the undetected presence of an inactive *Pgm-2* allele was examined and found to be inconsistent with all of its predicted effects on the specific activity data. Genuine overdominance was shown to be capable of explaining the specific activities of ten structural locus genotypes, allelic frequency distributions in natural populations, and the maintenance of the enzyme polymorphism in a balanced state. The results provide evidence favoring the overdominance explanation for one locus involved in a heterozygosity/growth relationship and suggest that the reported effects of this locus on adult body weight may have been caused by the greater flux capacities of heterozygotes for the $Pgm\text{-}2^{100}$ allele.

DOCUMENTING the action of selection on naturally occurring enzyme polymorphisms requires a multidisciplinary approach incorporating elements of biochemistry, physiology, and ecology. The evidence needed to support a role for selection has been outlined by CLARKE (1975) and KOEHN (1978). Enzyme genotypes must first be demonstrated to possess differing catalytic, regulatory or structural properties. These biochemical differences must then be shown to directly affect relevant physiological processes which, through their interaction with environmental factors, ultimately lead to differences in relative fitness between phenotypes. Although many studies have described the existence of biochemical differences between allozymes, few have proceeded beyond this point to examine the functional significance of the observed variation. However, convincing evidence favoring selection has been obtained in several instances, notably at the aminopeptidase-1 locus in the mussel, *Mytilus edulis* (reviewed by KOEHN and HILBISH 1987), the *Pgi* locus in Colias butterflies (reviewed by WATT 1985), and the *Ldh-B* locus in the killifish, *Fundulus heteroclitus* (reviewed by POWERS, DIMICHELE and PLACE 1983). Through their mechanistic linking of genotype with phenotype, these studies have provided important insights into the adaptable properties of enzymes and the environmental factors responsible for maintaining allozymic variation in nature.

The direct study of enzyme polymorphisms also has the potential to discriminate between hypotheses which have been advanced to explain correlations between multiple-locus heterozygosity and fitness-related traits in natural populations (reviewed by MITTON and GRANT 1984; ZOUROS and FOLTZ 1987). As described in detail elsewhere (ZOUROS, ROMERO-DOREY and MALLET 1988; ZOUROS and MALLET 1989), the critical distinction between these competing explanations concerns the role played by the electrophoretic loci scored. Three selection-based hypotheses [true overdominance, multiple-locus dominance, and the balanced pathway hypothesis of KOEHN, DIEHL and SCOTT (1988)], along with two founded on the presence of inactive enzyme alleles (FOLTZ 1986; CHAKRABORTY 1989) predict the direct involvement of the polymorphic loci. In contrast, aneuploidy (*e.g.* THIRIOT-QUIÉVREUX 1986) and associative overdominance (*cf.* OHTA 1971) both treat the enzyme loci as neutral markers; the former ascribing the heterozygosity relationship to the loss of many

functional genes, the latter to the effects of tightly linked loci segregating for deleterious recessive alleles. Direct study of the electrophoretic loci may represent the most powerful means of distinguishing between these alternatives because all "direct involvement" explanations predict that some measurable biochemical differences exist between enzyme genotypes. Although the failure to detect such variation does not necessarily eliminate the involvement of the enzyme locus, any positive evidence provided by this approach severely weakens the need to invoke hypotheses that rely on the contribution of linked genes of unknown function.

A direct test of the role played by selection has been taken with the phosphoglucomutase-2 locus (*Pgm-2*) in the Pacific oyster, *Crassostrea gigas*, one of five loci involved in a significant positive correlation between multiple-locus heterozygosity and adult body weight described by FUJIO (1982). In a previous report (POGSON 1989) it was shown that kinetic and structural differences exist between *Pgm-2* genotypes, but appear unable to account for the larger body weights of heterozygous individuals. This paper examines the potential contribution of differences between the specific enzyme activities of *Pgm-2* genotypes. Specific activity variation has been frequently described between genotypes at polymorphic enzyme loci (see reviews in HARRIS 1975; LAURIE-AHLBERG 1985; ZERA, KOEHN and HALL 1985), and in several instances has been linked to the expression of differences in physiological performance or partial fitness components (*e.g.*, HICKEY 1979; VAN DELDEN 1982; BURTON and FELDMAN 1983). The results presented here show that specific activity differences are present between *Pgm-2* genotypes in a pattern consistent with the overdominance explanation: the three most common *Pgm-2* heterozygotes display the unusual property of expressing larger specific activities than their respective homozygotes.

MATERIALS AND METHODS

Animals: Oysters were collected in the summer (late June), fall (early November), and winter (early March) from a seminatural population located in Nanoose Bay on Southeastern Vancouver Island, British Columbia. Samples consisted of 150–250 mature oysters ranging in size from 5–20-cm shell length from two intertidal locations (designated as "low" and "high" water). Animals were returned to the laboratory on ice, excised from their shells, weighed, and after removal of a small section of mantle for electrophoresis, frozen at −40°.

Electrophoresis: *Pgm-2* genotype was identified by horizontal starch gel electrophoresis employing a Tris-borate-EDTA (pH 8.3) buffer system. Details of the electrophoretic procedure have been described previously (POGSON 1989).

Specific activity measurements: Oysters were divided into four arbitrarily assigned body weight classes (12.0–23.9 g; 24.0–35.9 g; 36.0–47.9 g; ≥48.0 g). Specific activities of the four most common genotypes (*Pgm-2*$^{92/100}$, *Pgm-2*$^{96/100}$, *Pgm-2*$^{100/104}$ and *Pgm-2*$^{100/100}$) were determined on a subsample of 3–6 individuals randomly selected from each group. Owing to their rarity, all homozygotes for the less frequent *Pgm-2*^{92}, *Pgm-2*^{96} and *Pgm-2*^{104} alleles (and heterozygotes for these alleles examined in the fall) were included for study, irrespective of body weight.

Phosphoglucomutase (PGM) activity was measured in the mantle and posterior adductor muscle tissues of selected genotypes. From the mantle, approximately 1 g of tissue was dissected from the most posterior region of the left lobe, weighed and homogenized in 5 ml of ice-cold extraction buffer (10 mM Tris, 10 mM maleic acid, 1 mM $MgCl_2$, 1 mM EDTA, pH 7.4). Similarly, a 0.5-g section of the "quick" portion of the posterior adductor muscle was dissected, weighed and homogenized in 3.5 ml of buffer. After centrifuging at 12,000 × *g* for 20 min, a 1-ml aliquot of supernatant was removed for the measurement of PGM activity and soluble protein.

PGM activity was determined at 15° in the forward reaction direction at 340 nm on a Pye Unicam SP 1800 UV/visible spectrophotometer. The assay medium contained 50 mM imidazole-HCl, 3 mM $MgCl_2$, 2 mM glucose-1-phosphate, 16 μM glucose-1,6-diphosphate, 0.4 mM NADP, 1 unit glucose-6-phosphate dehydrogenase, pH 7.0 (20°), in a final volume of 1 ml. One unit of activity is defined as the quantity of enzyme required to convert 1 μmol of glucose-1-phosphate to glucose-6-phosphate per minute under the above conditions. All enzyme assays were performed in triplicate and the samples frozen at −70° prior to the determination of soluble protein. General protein was measured in triplicate at room temperature by the method of BRADFORD (1976) on a Pye Unicam SP8-400 UV/visible spectrophotometer using γ-globulin as a standard. Specific activity is expressed in units of PGM activity/mg protein in the crude homogenates. PGM activity/g wet tissue and soluble protein extracted/g wet tissue were calculated by assuming an intracellular water content of 84% in the mantle and 80% in the adductor muscle tissue (WALSH, MCDONALD and BOOTH 1984).

For each seasonal sample, specific activities were determined following a set of guidelines that minimized day-to-day variability in extraction and/or assay techniques. First, of the 12 individuals examined each day, equal numbers were selected from both intertidal locations. Second, a minimum of four different genotypes were studied daily, thus ensuring a maximum representation of three individuals of the same genotype. Third, the order in which genotypes were assayed for PGM activity and soluble protein was completely randomized. These precautions ensured that the specific activity measurements for all genotypes were suitably randomized and completed within similar time periods.

Statistical analyses: The specific activity data were analyzed each season by three-factor ANOVAs (SOKAL and ROHLF 1981) treating intertidal position, *Pgm-2* genotype and body weight class as independent variables. Body weight was found to exert minor effects on PGM specific activity in both the mantle and adductor muscle tissues and hence was eliminated from further analyses. Homogeneity of variance tests indicated that the soluble protein data required log transformation to normalize variances. All means were compared by *a posteriori* Bonferroni multiple range tests.

RESULTS

A summary of the ANOVAs on PGM specific activity and its component variables are presented in Table 1. Enzyme activity levels, standardized against soluble

TABLE 1

F* ratios from the analyses of variance on PGM specific activity in the mantle and adductor muscle tissues of *C. gigas

Source of variation	d.f.	Mantle			Adductor muscle		
		Specific activity[a]	PGM activity[b]	Soluble protein[c]	Specific activity[a]	PGM activity[b]	Soluble protein[c]
Season	2	39.3***	81.7***	77.9***	271.3***	167.1***	23.0***
Tidal height	1	2.52	13.3***	5.48*	5.56*	6.75**	3.46
Genotype	6	11.9***	9.60***	2.62*	21.9***	12.8***	1.40
Genotype × season	12	1.34	0.83	0.54	1.09	0.53	0.50
Genotype × tidal height	6	0.54	1.29	0.24	0.84	1.06	0.40
Season × tidal height	2	23.8***	17.8***	0.35	13.7***	5.12**	3.73*
Genotype × tidal height × season	12	0.74	0.80	1.14	1.20	0.52	0.37
Error	456						

[a] Units/mg protein.
[b] Units/g wet tissue.
[c] Milligrams protein/g wet tissue. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

protein or wet tissue weight, differed significantly between intertidal locations in all three seasons. Non-additive changes in enzyme activity between the fall and winter samples produced significant season-by-tidal height interactions (Figure 1), but no interactions occurred between these variables and *Pgm-2* genotype. Soluble protein also fluctuated significantly between seasons but exerted little effect on the observed variation in specific activity.

Significant differences were observed between *Pgm-2* genotypes in all three seasons (Table 2). *Pgm-2* heterozygotes consistently expressed greater specific activities than their respective homozygotes, although multiple range tests comparing individual genotypes were often inconclusive. Analysis of the pooled data, however, yielded highly significant results and clarified these relationships (Table 3). In both tissues the three heterozygotes and the four homozygotes behaved as homogeneous groups. In the adductor muscle the specific activities of the $Pgm\text{-}2^{92/100}$, $Pgm\text{-}2^{96/100}$ and $Pgm\text{-}2^{100/104}$ heterozygotes significantly exceeded all four homozygotes; in the mantle they differed significantly from all homozygotes except $Pgm\text{-}2^{92/92}$. The magnitude of this overdominance was similar in both cases: the specific enzyme activities of heterozygotes surpassed homozygotes by 24% in the mantle and by 20% in the adductor muscle.

Table 3 shows that the elevated specific activities of *Pgm-2* heterozygotes were mediated through their increased enzyme activity levels (29% and 24% larger than homozygotes in the mantle and adductor muscle, respectively). This overdominance was not restricted to enzyme activity, however, since in both tissues heterozygotes also displayed slightly higher concentrations of soluble protein than homozygotes.

Due to the high frequency of the $Pgm\text{-}2^{100}$ allele in the study population (see POGSON 1989), the $Pgm\text{-}2^{92/100}$, $Pgm\text{-}2^{96/100}$ and $Pgm\text{-}2^{100/104}$ genotypes represent approximately 75% of all heterozygotes at this locus. The next most frequent group of heterozygotes are formed between the three rarer *Pgm-2* alleles; $Pgm\text{-}2^{92/96}$, $Pgm\text{-}2^{92/104}$ and $Pgm\text{-}2^{96/104}$. To determine if these heterozygotes also displayed overdominant specific activities, they were included for study in the fall sample. Table 4 summarizes the data from this season, pooled across intertidal positions, for two classes of homozygotes and heterozygotes which have been grouped according to the presence or absence of the $Pgm\text{-}2^{100}$ allele. In sharp contrast to heterozygotes for the $Pgm\text{-}2^{100}$ allele, those lacking this allele expressed enzyme activities and soluble protein levels that did not differ significantly from either homozygote group. Therefore, overdominance was not consistently expressed at the *Pgm-2* locus; heterozygotes for the $Pgm\text{-}2^{92}$, $Pgm\text{-}2^{96}$ and $Pgm\text{-}2^{104}$ alleles appeared equivalent to homozygotes and, if anything, exhibited a tendency to display underdominance.

DISCUSSION

This study has demonstrated that phosphoglucomutase activity in the mantle and adductor muscle tissues of *C. gigas* is significantly affected by the season of collection, intertidal location, and *Pgm-2* genotype. The significant contributions of the first two factors are not unexpected since enzyme activity levels in marine bivalves often exhibit pronounced seasonal fluctuations (*e.g.*, GABBOTT and HEAD 1980; LIVINGSTONE and CLARKE 1983), indicative of prevailing environmental conditions and the state of their annual reproductive cycle (LIVINGSTONE 1981; GABBOTT 1983). The expression of overdominant PGM activities by oysters heterozygous for the $Pgm\text{-}2^{100}$ allele is, however, unusual. When differences in specific enzyme activity have been recorded between genotypes at polymorphic enzyme loci, heterozygote intermediacy is almost invariably observed (*e.g.*, GILLESPIE and

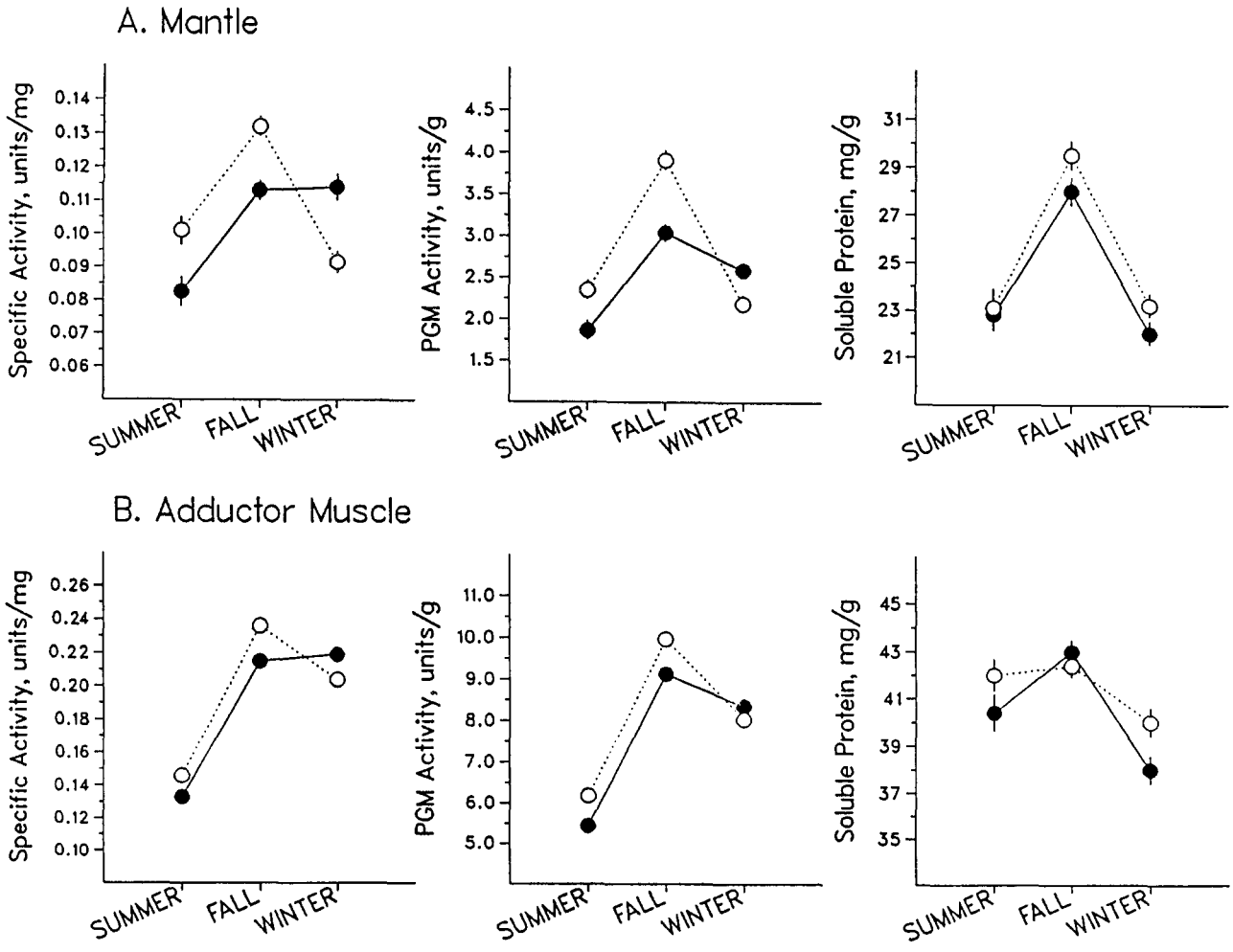

FIGURE 1.—Effects of season and intertidal position on specific activity, PGM activity and soluble protein. Open circles = low intertidal (summer, $N = 64$; fall, $N = 96$; winter, $N = 89$), closed circles = high intertidal (summer, $N = 57$; fall, $N = 100$; winter, $N = 92$). Bars represent ±1 standard error where visible or fall within the plotted symbol.

LANGLEY 1974; HARRIS 1975), although dominance has sometimes been described (*e.g.*, GIBSON *et al.* 1986; KING and MCDONALD 1987).

Overdominance for enzyme activity has been reported only in exceptional circumstances. For example, WHALEY (1952) stated that maize F1 hybrids expressed greater catalase activities in their meristems than their inbred parental lines, and DICKINSON, ROWAN and BRENNAN (1984) observed that interspecific hybrids between *Drosophila melanogaster* and *D. simulans* exhibited greater ADH activities than either parental species. Heterozygotes at an esterase locus in two fresh-water fish species have been shown to display greater activities than homozygotes at some temperatures but not at others (KOEHN 1969; KOEHN, PEREZ and MERRITT 1971). WATT (1977, 1983) has documented that several PGI heterozygotes in Colias butterflies possess overdominant V_{max}/K_m ratios, but these were produced by differences in K_m, not enzyme activity. In the amphipod *Gammarus insensibilis*, PATARNELLO, BISOL and BATTAGLIA (1989) recently reported that the most common heterozygote at the PGI locus expressed overdominant activity levels, but their results are complicated by the fact that assays were performed on pooled individuals. The overdominance for specific activity at the *Pgm-2* locus reported in the present study appears unique in its clarity of expression, reproducibility across different seasons, intertidal positions and tissues, and its insensitivity to environmental factors.

The unusual behavior of *Pgm-2* heterozygotes cannot be explained as an artifact of measurement technique. Care was taken to randomize the selection of *Pgm-2* genotypes within and across days, thus minimizing the occurrence of systematic errors. Examination of the spontaneous loss of PGM activity over the measurement periods showed it to be minimal (<10%) and not to differ significantly between genotypes (G. H. POGSON, unpublished data). One important complication does arise from the assay of PGM activity in crude homogenates due to the contribution of enzyme from the more cathodal, polymorphic *Pgm-1* locus. However, there are two reasons why the *Pgm-1* locus is unlikely to have had any significant impact on the

TABLE 2

Seasonal variation in the specific enzyme activities of seven *Pgm-2* genotypes

Pgm-2 genotype	*N*	Activity[a] at season: Summer	*N*	Fall	*N*	Winter
A. Mantle						
92/92	5	0.085 ± 0.015	8	0.128 ± 0.011	9	0.085 ± 0.010
92/100	23	0.111 ± 0.007	41	0.129 ± 0.005	35	0.115 ± 0.005
96/96	4	0.082 ± 0.016	7	0.113 ± 0.011	11	0.066 ± 0.009
96/100	25	0.085 ± 0.006	42	0.129 ± 0.005	31	0.112 ± 0.005
100/100	29	0.086 ± 0.006	42	0.109 ± 0.005	40	0.091 ± 0.005
100/104	23	0.100 ± 0.007	40	0.128 ± 0.005	40	0.119 ± 0.005
104/104	12	0.078 ± 0.009	16	0.109 ± 0.008	15	0.079 ± 0.008
		$F_{(6,77)} = 2.42$*		$F_{(6,144)} = 3.09$**		$F_{(6,132)} = 8.23$***
B. Adductor muscle						
92/92	5	0.127 ± 0.013	8	0.219 ± 0.013	9	0.176 ± 0.010
92/100	23	0.155 ± 0.006	41	0.233 ± 0.006	35	0.230 ± 0.005
96/96	4	0.130 ± 0.014	7	0.219 ± 0.014	11	0.175 ± 0.009
96/100	25	0.146 ± 0.006	42	0.236 ± 0.006	31	0.226 ± 0.006
100/100	29	0.124 ± 0.005	42	0.206 ± 0.006	40	0.191 ± 0.005
100/104	23	0.153 ± 0.006	40	0.238 ± 0.006	40	0.233 ± 0.005
104/104	12	0.121 ± 0.008	16	0.204 ± 0.009	15	0.181 ± 0.008
		$F_{(6,77)} = 4.93$***		$F_{(6,144)} = 4.50$***		$F_{(6,132)} = 15.0$***

[a] Units/mg protein. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 3

Separation of *Pgm-2* specific activity into enzyme activity and soluble protein expressed on a tissue weight basis

Pgm-2 genotype	*N*	Mantle: Specific activity[a]	PGM activity[b]	Soluble protein[c]	Adductor muscle: Specific activity[a]	PGM activity[b]	Soluble protein[c]
92/92	22	0.101 ± 0.006	2.46 ± 0.22	23.7 ± 0.83	0.181 ± 0.007	7.40 ± 0.42	40.5 ± 1.00
92/100	99	0.120 ± 0.003	3.09 ± 0.10	25.5 ± 0.52	0.214 ± 0.003	8.62 ± 0.19	41.0 ± 0.53
96/96	22	0.084 ± 0.006	2.07 ± 0.21	23.2 ± 0.98	0.181 ± 0.007	7.21 ± 0.47	39.9 ± 1.14
96/100	98	0.112 ± 0.003	2.90 ± 0.13	25.9 ± 0.53	0.210 ± 0.003	8.78 ± 0.24	42.1 ± 0.57
100/100	111	0.097 ± 0.003	2.47 ± 0.11	24.9 ± 0.49	0.179 ± 0.003	7.31 ± 0.23	40.1 ± 0.55
100/104	103	0.118 ± 0.003	3.07 ± 0.09	25.9 ± 0.51	0.217 ± 0.003	8.79 ± 0.23	41.2 ± 0.52
104/104	43	0.090 ± 0.005	2.06 ± 0.15	22.6 ± 0.79	0.173 ± 0.005	7.01 ± 0.34	40.6 ± 0.83
		$F_{(6,456)} = 1.9$***	9.60***	2.62*	21.9***	12.8***	1.40

[a] Units/mg protein.
[b] Units/g wet tissue.
[c] Milligrams protein/g wet tissue. * $P < 0.05$; *** $P < 0.001$.

results. First, it remained unscorable in both tissues throughout the study due to its low level of activity. Second, the *Pgm-1* locus segregates independently from *Pgm-2* in *C. gigas* (WILKINS 1976; G. H. POGSON, unpublished data), thus diminishing its predicted effect to a small, unquantified level of background activity equally represented in all *Pgm-2* genotypic classes.

Differences between the kinetic properties of *Pgm-2* allozymes appear unable to explain the nonadditive behavior of heterozygotes. Functional variation between the four most common allozymes is limited and, more importantly, heterozygotes for the $Pgm\text{-}2^{100}$ allele exhibit strictly intermediate temperature stabilities, apparent Michaelis constants for glucose-1-phosphate and glucose-1,6-diphosphate, and V_{max}/K_m ratios over broad ranges of temperature and pH (POGSON 1989). These results suggest that differences in specific activity observed between *Pgm-2* genotypes reflect differences in steady-state enzyme concentrations.

One factor that may have directly influenced enzyme concentration is the undetected presence of a *Pgm-2* null allele. FOLTZ (1986) has observed the segregation of inactive alleles at two loci (*Lap* and *Mpi*) in the American oyster, *Crassostrea virginica*, and KATOH and FOLTZ (1987) reported that *Lap* null heterozygotes possess about 60% of the enzyme activity of a normal genotype. If a null allele exists at the *Pgm-2* locus in *C. gigas*, null heterozygotes would have been erroneously scored and pooled unknowingly into the four homozygote classes. This would have had the combined effects of (1) lowering the specific activities of homozygotes relative to heterozygotes with two

TABLE 4

Comparison of the enzyme activities and soluble protein levels of homozygotes and heterozygotes possessing or lacking the *Pgm-2*^{100} allele

Genotypic class	*N*	Mantle			Adductor muscle		
		Specific activity[a]	PGM activity[b]	Soluble protein[c]	Specific activity[a]	PGM activity[b]	Soluble protein[c]
Homozygotes for *100* allele	42	0.109 ± 0.005	3.10 ± 0.16	28.4 ± 0.86	0.206 ± 0.006	8.83 ± 0.28	42.6 ± 0.75
Homozygotes without *100* allele	31	0.117 ± 0.005	3.00 ± 0.19	26.1 ± 1.00	0.211 ± 0.007	8.90 ± 0.33	42.2 ± 0.86
Heterozygotes for *100* allele	123	0.129 ± 0.003	3.70 ± 0.10	29.2 ± 0.50	0.236 ± 0.003	9.97 ± 0.16	42.5 ± 0.44
Heterozygotes without *100* allele	33	0.105 ± 0.005	2.65 ± 0.18	24.9 ± 0.98	0.198 ± 0.006	8.53 ± 0.32	42.9 ± 0.85
		$F_{(3,197)} = 7.76$***	8.81***	6.71***	12.9***	7.79***	0.84

[a] Units/mg protein.
[b] Units/g wet tissue.
[c] Milligrams protein/g wet tissue.
*** $P < 0.001$.

functional alleles and (2) generating deficiencies of heterozygotes in proportion to the frequency of the null. Based on the magnitude of the heterozygote deficiencies observed at this locus ($\overline{D} = -0.076$; POGSON 1989) it is possible to place an upper limit on the frequency of the putative null at 0.044 and test a number of its predicted effects on the specific activity data.

One important consequence of scoring null heterozygotes as homozygotes is that it should differentially affect the specific activities of the four homozygote classes. These differences are expected because the proportion of null heterozygotes represented in each homozygote group would vary inversely with the frequency of the *Pgm-2* functional allele (this being $2pn/(p^2 + 2pn)$, where n is the frequency of the null and p is the *Pgm-2* allozyme frequency). Assuming a null frequency of 0.044, only 13% of genotypes classified as homozygotes for the most common *Pgm-2*^{100} allele are expected to be null heterozygotes, whereas in the three other homozygote groups the percentage of null heterozygotes should be considerably larger (approximately 37, 42 and 49% for the *Pgm-2*$^{104/104}$, *Pgm-2*$^{92/92}$ and *Pgm-2*$^{96/96}$ classes, respectively). As a result, *Pgm-2*$^{100/100}$ homozygotes are predicted to closely resemble heterozygotes for the *Pgm-2*^{100} allele and exhibit significantly larger specific activities than the other homozygotes. This strong dichotomy expected between *Pgm-2* homozygotes, which should hold irrespective of the null's actual frequency, is not reflected in the specific activity data (see Table 2).

In contrast to the frequency-dependent effects of a null allele, the *de novo* arisal of inactive *Pgm-2* alleles by molecular imprinting (CHAKRABORTY 1989), or their loss through aneuploidy, are not constrained to affect one homozygote group more than another. However, all are expected to inflate the variance of the specific activity measurements in homozygotes and cause the production of bimodal activity distributions (corresponding to heterozygotes for the nonfunctional/lost allele and normal homozygotes). Homogeneity of variance tests detected no differences between the variances of *Pgm-2* genotypes and visual inspection of the normalized activity distributions failed to discern any sign of this expected bimodality. An inactive *Pgm-2* allele also cannot account for the greater soluble protein levels displayed by heterozygotes for the *Pgm-2*^{100} allele. Since PGM enzyme has been estimated to represent only 1% of the total intracellular protein pool (CZOK and BUCHER 1960; OTTAWAY and MOWBRAY 1977), no differences in soluble protein should have been observed between *Pgm-2* genotypes. Only aneuploidy, through the simultaneous loss of many functional genes, can explain the larger soluble protein levels expressed by *Pgm-2* heterozygotes.

Irrespective of mechanism, all of the above explanations that rely on the reduction of PGM activity in homozygotes to account for the observed overdominance are severely compromised by the relative performance of the two heterozygote groups examined in the fall (Table 4). Here, heterozygotes for the *Pgm-2*^{92}, *Pgm-2*^{96} and *Pgm-2*^{104} alleles did not express greater enzyme activities or soluble protein concentrations than either homozygote class. If an inactive or lost *Pgm-2* allele is responsible for depressing the enzyme activities of genotypes scored as homozygotes, no significant differences are expected between heterozygotes that possess two functional alleles. The observation that homozygotes and heterozygotes for the three less frequent *Pgm-2* alleles are indistinguishable strongly contradicts these explanations and shows that heterozygosity *per se* is not sufficient for the

expression of these unusual properties. Instead, a particular allelic configuration is required; the *Pgm-2*^{100} allele must be paired with either the *Pgm-2*^{92}, *Pgm-2*^{96} or *Pgm-2*^{104} alleles before the overdominant effects are manifested.

An alternative explanation for these results is that tightly linked to, or associated with, the *Pgm-2* structural locus is an overdominant regulatory locus (*cf.* PAIGEN 1979) that produces greater steady-state PGM activity levels in heterozygotes relative to homozygotes. If a regulatory element is involved, however, its behavior is unlike any previously characterized example. The general pattern that has emerged from studies on regulatory polymorphisms in eukaryotes is that both *trans*-acting (*e.g.*, SCANDALIOS *et al.* 1980; DOANE *et al.* 1983) and *cis*-acting (*e.g.*, DICKINSON 1975; SHAFFER and BEWLEY 1983) variants that affect rates of transcription produce intermediate enzyme activities in heterozygotes, while those acting post-translationally tend to be inherited in a dominant/recessive fashion (*e.g.*, LAI and SCANDALIOS 1980; KING and MCDONALD 1983, 1987). The regulatory polymorphism envisaged here must be *trans*-acting, but function in a distinctly nonadditive fashion in heterozygous condition.

If fitness is correlated with enzyme activity at the *Pgm-2* locus, this overdominance model can produce a genotypic fitness array consistent with the maintenance of a stable polymorphic equilibrium. Based on the data summarized in Table 4 it is possible to group the *Pgm-2* genotypes into three phenotypic classes corresponding to their hypothesized regulatory genotype. According to this model, regulatory variant "A" exists in complete disequilibrium with the *Pgm-2*^{100} structural allele. Associated with the *Pgm-2*^{92}, *Pgm-2*^{96} and *Pgm-2*^{104} alleles is regulatory variant "B." Homozygotes for the A allele produce only one genotype, *Pgm-2*$^{100/100}$. Heterozygotes for the A and B alleles give rise to an overdominant phenotype manifested in the *Pgm-2*$^{92/100}$, *Pgm-2*$^{96/100}$ and *Pgm-2*$^{100/104}$ genotypes. Homozygotes for the B allele yield six phenotypically equivalent *Pgm-2* genotypes comprising both homozygotes and heterozygotes for the *Pgm-2*^{92}, *Pgm-2*^{96} and *Pgm-2*^{104} alleles. This interpretation can explain the similarities observed between ten structural locus genotypes and, by collapsing the multiallelic system into a two-allele regulatory polymorphism, eliminates the difficulties associated with maintaining stable multiallelic polymorphisms by overdominance (*e.g.*, LEWONTIN, GINZBURG and TULJAPURKAR 1978). This explanation also predicts that it is the combined frequencies of the less frequent *Pgm-2* alleles relative to *Pgm-2*^{100} that control the dynamics of the polymorphism. Support for this assertion comes from the observation that the frequency of *Pgm-2*^{100} is remarkably invariant across wide geographic areas but those of the less frequent *Pgm-2* alleles are not (*e.g.*, BUROKER, HERSHBERGER and CHEW 1979; OZAKI and FUJIO 1985).

The overdominance observed at the *Pgm-2* locus in *C. gigas* has a number of important implications for studies examining the associations between multiple-locus heterozygosity and phenotypic traits. As described for the *Pgi* locus in Colias butterflies by WATT (1977, 1983), it is one of the few cases in which overdominance has been detected at a polymorphic enzyme locus, and it is the first time it has been observed at one involved in a heterozygosity relationship (*cf.* FUJIO 1982). The expression of overdominant specific activities by the three most common *Pgm-2* heterozygotes has the potential to account for the phenotypic effects attributed to this locus, thereby eliminating the need for neutral explanations which discount any effect of the electrophoretic locus itself. Another important finding concerns the distinctive properties of different *Pgm-2* heterozygotes. In heterozygosity studies it is common to pool all homozygotes and heterozygotes together, implicitly assuming that the genotypes combined within these groups are equivalent. The clear absence of overdominant effects in heterozygotes for the three less frequent *Pgm-2* alleles suggests that a sizable amount of error may be introduced by this assumption since, in the present case, these genotypes represent approximately 24% of all *Pgm-2* heterozygotes.

The correspondence between the biochemical and phenotypic effects of heterozygosity at the *Pgm-2* locus is striking, especially in light of theoretical treatments of the impact of minor fluctuations in enzyme activity levels on metabolic flux (*e.g.*, KACSER and BURNS 1981; HARTL, DYKHUIZEN and DEAN 1985). However, the overdominant specific activities of heterozygotes for the *Pgm-2*^{100} allele automatically imparts upon these genotypes larger catalytic capacities than all other *Pgm-2* genotypes. Situated at the glucose-6-phosphate branch point, these differences have the potential to directly affect the partitioning of flux between glycolysis, the pentose shunt, and the synthesis of glycogen. To strengthen the link between this enzyme activity variation and differential rates of growth, it will be necessary to examine the physiological effects of this polymorphism on the metabolism of glycogen, the biochemical pathway in which PGM functions.

Helpful comments on the manuscript were provided by C. F. WEHRHAHN, E. ZOUROS and E. B. TAYLOR. Funding for the study was provided by NSERC operating grants to C. F. WEHRHAHN and an NSERC Postgraduate Scholarship to G.H.P.

LITERATURE CITED

BRADFORD, M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the

principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

BUROKER, N. E., W. K. HERSHBERGER and K. K. CHEW, 1979 Population genetics of the family Ostreidae. I. Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis*. Mar. Biol. **54:** 157–169.

BURTON, R. S., and M. W. FELDMAN, 1983 Physiological effects of an allozyme polymorphism: glutamate-pyruvate transaminase and response to hyperosmotic stress in the copepod *Tigriopus californicus*. Biochem. Genet. **21:** 239–251.

CLARKE, B., 1975 The contribution of ecological genetics to evolutionary theory: detecting the direct effects of natural selection on particular polymorphic loci. Genetics **79:** 101–113.

CHAKRABORTY, R., 1989 Can molecular imprinting explain heterozygote deficiency and hybrid vigor? Genetics **122:** 713–717.

CZOK, R., and TH. BUCHER, 1960 Crystallized enzymes from the myogen of rabbit skeletal muscle. Adv. Protein Chem. **15:** 315–415.

DICKINSON, W. J., 1975 A genetic locus affecting the developmental expression of an enzyme in *Drosophila melanogaster*. Dev. Biol. **42:** 131–140.

DICKINSON, W. J., R. G. ROWAN and M. D. BRENNAN, 1984 Regulatory gene evolution: adaptive differences in expression of alcohol dehydrogenases in *Drosophila melanogaster* and *Drosophila simulans*. Heredity **52:** 215–225.

DOANE, W. W., L. G. TREAT-CLEMONS, R. M. GEMMIL, J. N. LEVY, S. A. HAWLEY, A. M. BUCHBERG and K. PAIGEN, 1983 Genetic mechanism for the tissue-specific control of alpha-amylase expression in *Drosophila melanogaster*. Isozymes Curr. Top. Biol. Med. Res. **9:** 63–90.

FOLTZ, D. W., 1986 Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica*. Evolution **40:** 869–870.

FUJIO, Y., 1982 A correlation of heterozygosity with growth rate in the Pacific oyster, *Crassostrea gigas*. Tohoku J. Agric. Res. **33:** 66–75.

GABBOTT, P. A., 1983 Developmental and seasonal metabolic activities in marine molluscs, pp. 165–217 in *The Mollusca, Vol. 2. Environmental Biochemistry and Physiology*, edited by P. W. HOCHACHKA. Academic Press, New York.

GABBOTT, P. A., and E. J. H. HEAD, 1980 Seasonal changes in the specific activities of the pentose phosphate cycle enzymes, G6PDH and 6PGDH, and NADP-dependent isocitrate dehydrogenase in the bivalves *Mytilus edulis*, *Ostrea edulis* and *Crassostrea gigas*. Comp. Biochem. Physiol. **66B:** 279–284.

GIBSON, J. B., A. V. WILKS, A. CAO and A. L. FREETH, 1986 Dominance for *sn*-glycerol-3-phosphate dehydrogenase activity in *Drosophila melanogaster*: evidence for differential allelic expression mediated via a *trans*-acting effect. Heredity **56:** 227–235.

GILLESPIE, J. H., and C. H. LANGLEY, 1974 A general model to account for enzyme variation in natural populations. Genetics **76:** 837–848.

HARRIS, H., 1975 *Principles of Human Biochemical Genetics*. North Holland, Amsterdam.

HARTL, D. L., D. E. DYKHUIZEN and A. M. DEAN, 1985 Limits of adaptation: the evolution of selective neutrality. Genetics **111:** 655–674.

HICKEY, D. A., 1979 Selection on amylase allozymes in *Drosophila melanogaster*: selection experiments using several independently derived pairs of chromosomes. Evolution **33:** 1128–1137.

KACSER, H., and J. A. BURNS, 1981 The molecular basis of dominance. Genetics **97:** 639–666.

KATOH, M., and D. W. FOLTZ, 1987 Leucine aminopeptidase specific activity is significantly reduced in *Lap* null heterozygous oysters (*Crassostrea virginica*). Genetics **116** (Suppl.): s44.

KING, J. J., and J. F. MCDONALD, 1983 Genetic localization and biochemical characterization of a *trans*-acting regulatory effect in Drosophila. Genetics **105:** 55–69.

KING, J. J., and J. F. MCDONALD, 1987 Post-translational control of alcohol dehydrogenase levels in *Drosophila melanogaster*. Genetics **115:** 693–699.

KOEHN, R. K., 1969 Esterase heterogeneity: dynamics of a polymorphism. Science **163:** 943–944.

KOEHN, R. K., 1978 Physiology and biochemistry of enzyme variation: the interface between ecology and population genetics, pp. 51–72 in *Ecological Genetics: The Interface*, edited by P. F. BRUSSARD. Springer-Verlag, New York.

KOEHN, R. K., W. J. DIEHL and T. M. SCOTT, 1988 The differential contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam, *Mulinia lateralis*. Genetics **118:** 121–130.

KOEHN, R. K., and T. J. HILBISH, 1987 The adaptive significance of genetic variation. Am. Sci. **75:** 134–141.

KOEHN, R. K., J. E. PEREZ and R. B. MERRITT, 1971 Esterase enzyme function and genetical structure of populations of the freshwater fish, *Notropis stramineus*. Am. Nat. **105:** 51–69.

LAI, Y.-K., and J. G. SCANDALIOS, 1980 Genetic determination of the developmental program for maize scutellar alcohol dehydrogenase: involvement of a recessive, *trans*-acting, temporal regulatory gene. Dev. Genet. **1:** 311–324.

LAURIE-AHLBERG, C. C., 1985 Genetic variation affecting the expression of enzyme coding genes in *Drosophila*: an evolutionary perspective. Isozymes Curr. Top. Biol. Med. Res. **12:** 33–88.

LEWONTIN, R. C., L. R. GINZBURG and S. D. TULJAPURKAR, 1978 Heterosis as an explanation for large amounts of genic polymorphism. Genetics **88:** 149–170.

LIVINGSTONE, D. R., 1981 Induction of enzymes as a mechanism for the seasonal control of metabolism in marine invertebrates: glucose-6-phosphate dehydrogenases from the mantle and hepatopancreas of the common mussel *Mytilus edulis*. Comp. Biochem. Physiol. **69B:** 147–156.

LIVINGSTONE, D. R., and K. R. CLARKE, 1983 Seasonal changes in hexokinase from the mantle tissue of the common mussel *Mytilus edulis* L. Comp. Biochem. Physiol. **74B:** 691–702.

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

OHTA, T., 1971 Associative overdominance caused by linked detrimental mutations. Genet. Res. **18:** 277–286.

OTTAWAY, J. H., and J. MOWBRAY, 1977 The role of compartmentalization in the control of glycolysis. Curr. Top. Cell. Regul. **12:** 107–208.

OZAKI, H., and Y. FUJIO, 1985 Genetic differentiation in geographical populations of the Pacific oyster (*Crassostrea gigas*) around Japan. Tohoku J. Agric. Res. **36:** 49–61.

PAIGEN, K., 1979 Genetic factors in developmental regulation, pp. 1–49 in *Physiological Genetics*, edited by J. G. SCANDALIOS. Academic Press, New York.

PATARNELLO, T., P. M. BISOL and B. BATTAGLIA, 1989 Studies on differential fitness of PGI genotypes with regard to temperature in *Gammarus insensibilis* (Crustacea: Amphipoda). Mar. Biol. **102:** 355–359.

POGSON, G. H., 1989 Biochemical characterization of genotypes at the phosphoglucomutase-2 locus in the Pacific oyster, *Crassostrea gigas*. Biochem. Genet. **27:** 571–589.

POWERS, D. A., L. DIMICHELE and A. R. PLACE, 1983 The use of enzyme kinetics to predict differences in cellular metabolism, developmental rate, and swimming performance between LDH-B genotypes of the fish, *Fundulus heteroclitus*. Isozymes Curr. Top. Biol. Med. Res. **10:** 147–170.

SCANDALIOS, J. G., D.-Y. CHANG, D. E. MCMILLIN, A. TSAFTARIS and R. H. MOLL, 1980 Genetic regulation of the catalase developmental program in maize scutellum: identification of a

temporal regulatory gene. Proc. Natl. Acad. Sci. USA **77:** 5360–5364.

SHAFFER, J. B., and G. C. BEWLEY, 1983 Genetic determination of *sn*-glycerol-3-phosphate dehydrogenase synthesis in *Drosophila melanogaster*. J. Biol. Chem. **258:** 10027–10033.

SOKAL, R. R., and F. J. ROHLF, 1981 *Biometry*, Ed. 2. W. H. Freeman, San Francisco.

THIRIOT-QUIÉVEUX, C., 1986 Etude de l'aneuploïdie dans différents naissains d'Ostreidae (Bivalvia). Genetica **106:** 225–231.

VAN DELDEN, W., 1982 The alcohol dehydrogenase polymorphism in *Drosophila melanogaster*: selection at an enzyme locus. Evol. Biol. **15:** 187–222.

WALSH, P. J., D. G. MCDONALD and C. E. BOOTH, 1984 Acid-base balance in the sea mussel, *Mytilus edulis*. II. Effects of hypoxia and air exposure on intracellular acid-base status. Mar. Biol. Lett. **5:** 359–369.

WATT, W. B., 1977 Adaptation at specific loci. I. Natural selection on phosphoglucose isomerase of *Colias* butterflies: biochemical and population aspects. Genetics **87:** 177–194.

WATT, W. B., 1983 Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* PGI polymorphism. Genetics **103:** 691–724.

WATT, W. B., 1985 Allelic isozymes and the mechanistic study of evolution. Isozymes Curr. Top. Biol. Med. Res. **12:** 89–132.

WHALEY, W. G., 1952 Physiology of gene action in hybrids, pp. 98–113 in *Heterosis*, edited by J. W. GOWEN. Hafner, New York.

WILKINS, N. P., 1976 Genic variability in marine Bivalvia: implications and applications in molluscan aquaculture, pp. 549–563 in *Proceedings of the 10th European Symposium Marine Biology, Vol. 1, Mariculture*, edited by G. PERSOONE and E. JASPERS. Universa, Wetteren, Belgium.

ZERA, A. J., R. K. KOEHN and J. G. HALL, 1985 Allozymes and biochemical adaptation, pp. 633–674 in *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, Vol. 10, edited by G. A. KERKUT and L. I. GILBERT. Pergamon, New York.

ZOUROS, E., and D. W. FOLTZ, 1987 The use of allelic isozyme variation for the study of heterosis. Isozymes Curr. Top. Biol. Med. Res. **13:** 1–59.

ZOUROS, E., and A. L. MALLET, 1989 Genetic explanations for the growth/heterozygosity correlation in marine mollusks, pp. 317–324 in *Reproduction, Genetics and Distributions of Marine Organisms, Proceedings of the 23rd European Marine Biology Symposium*, edited by J. S. RYLAND and P. A. TYLER. Olsen and Olsen, Fredensborg, Denmark.

ZOUROS, E., M. ROMERO-DOREY and A. L. MALLET, 1988 Heterozygosity and growth in marine bivalves: further data and possible explanations. Evolution **42:** 1332–1341.

Communicating editor: J. R. POWELL