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Authors
Snyder, TP
Ayala, FJ

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FREQUENCY-DEPENDENT SELECTION AT THE *PGM-1* LOCUS OF *DROSOPHILA PSEUDOOBSCURA*

THOMAS P. SNYDER* AND FRANCISCO J. AYALA

Department of Genetics, University of California, Davis, California 95616

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ABSTRACT

Frequency-dependent fitness was studied at the *Pgm-1* locus of *Drosophila pseudoobscura* with respect to two fitness components: rate of development and larva-to-adult survival. The *Pgm-1* locus is very polymorphic with only two alleles, $Pgm\text{-}1^{100}$ and $Pgm\text{-}1^{104}$, occurring at high frequencies. For each of these two alleles, 20 homozygous strains were obtained from a sample of 1,140 wild-inseminated females. First-instar larvae of the two genotypes were combined in a set of eight different frequencies: 0.0, 0.10, 0.25, 0.40, 0.60, 0.75, 0.90, and 1.0. Frequency-dependent fitness effects were observed for the two survival-related fitness components examined: larvae of the less common genotype develop faster and have a higher probability of survival than larvae of the more common genotype. The rate of survival at intermediate genotypic frequencies is similar to that in pure cultures. If selection acted solely as frequency-dependent effects on survival-related components of fitness, the equilibrium frequency of the $Pgm\text{-}1^{100}$ allele would be 0.615 for a two-genotype system, which fits an observed frequency range for this allele in nature between 0.55 and 0.71. Experimentally created linkage disequilibrium was excluded from the experiment by using a large number of independent strains. It is nevertheless possible that the frequency-dependent selection may not affect the *Pgm-1* locus *per se*, but may reflect a linkage disequilibrium present in the natural population. Even if this were the case, the frequency-dependent selection could affect the frequency of the *Pgm-1* alleles in nature.

THE importance of frequency-dependent selection in maintaining genetic polymorphisms has been discussed by a number of authors (*e.g.*, AYALA and CAMPBELL 1974; SPIESS 1977). However, relatively few studies in experimental population genetics have sought to detect frequency-dependent selection at allozymic loci. This is somewhat surprising as frequency-dependent selection has intuitive appeal as a selective mode in maintaining a stable polymorphism in which all genotypes may have equal fitnesses at equilibrium (KOJIMA and YARBROUGH 1967). The studies of KOJIMA and his co-workers (HUANG, SING and KOJIMA 1971; KOJIMA and TOBARI 1969; KOJIMA and YARBROUGH 1967) presented evidence for frequency-dependent selection at the *Est-6* and *Adh* loci of *Drosophila melanogaster*. MARINKOVIC and AYALA (1975) presented evidence that suggested frequency-dependent selection at the *Pgm-1* and *Me-2* loci of *D.*

* Present address: Department of Biological Sciences, Michigan Technological University, Houghton, Michigan 49931.

pseudoobscura, while YAMAZAKI (1971) failed to find evidence for frequency-dependent selection at the *Est-5* locus of *D. pseudoobscura*. MUKAI, WATANABE and YAMAGUCHI (1974), using balancer chromosomes, failed to find evidence of frequency-dependent selection at the *Adh*, *αGpdh*, and *Est-6* loci in *D. melanogaster*.

This paper reports a study of frequency-dependent selection at the sex-linked phosphoglucomutase, *Pgm-1*, locus (or genes closely linked to that locus) of *Drosophila pseudoobscura*. The polymorphism at this locus in *D. pseudoobscura* is dynamic, with seasonal oscillations in the frequencies of the two common alleles (DOBZHANSKY and AYALA 1973). The causes of these oscillations are unknown, but may be due to temperature or density effects. A previous study of fitness components associated with allozyme genotypes at the *Pgm-1* locus (SNYDER and AYALA, in preparation) demonstrated differences among genotypes in egg-to-adult survival that were influenced by temperature and density. The present study was conducted to assess the possible role of frequency-dependent selection in maintaining the polymorphism of the *Pgm-1* locus.

MATERIALS AND METHODS

Frequency dependence of larva-to-adult survival and of rate of development was assessed for mixed cultures of two homozygous genotypes at the *Pgm-1* locus. Lines were derived from 1,140 wild-inseminated females collected at Redwood City, San Mateo County, California, in early September, 1976. Sib-pair matings were made for four generations with the progenies of individual wild-caught females. Forty independent lines were chosen for the experiments, 20 lines having the female parent homozygous and the male parent hemizygous for the $Pgm\text{-}1^{100}$ allele and the other 20 lines having the female homozygous and the male hemizygous for the $Pgm\text{-}1^{104}$ allele. Each of the 40 lines was maintained separately by mass culture for three additional generations before starting the present experiment.

Single-generation chain crosses among the lines of a strain were conducted to supply the larvae used in this study. Groups of five virgin females were mated with five males from a different line (*e.g.*, $Pgm\text{-}1^{100}$ line 1 × line 2, line 2 × line 3, etc.) and were then permitted to oviposit on spoons of Drosophila medium for 24 hr. Thus, all experimental larvae carry two independently derived chromosomes and are genetically equivalent to wild flies with the desired genotype at the *Pgm-1* locus. First instar larvae were collected from these spoons for initiation of experimental vials.

Experimental cultures were established in eight frequency groups, with five replicates, at the initial frequencies of $Pgm\text{-}1^{100}$ larvae of 0.0, 0.10, 0.25, 0.40, 0.60, 0.75, 0.90, and 1.00. The remaining larvae were of the $Pgm\text{-}1^{104}$ genotype. There were 60 larvae per culture, a density sufficient to necessitate some competition in the 18-mm diameter vials without causing excessive crowding. Each vial contained 10 ml of standard cornmeal-agar-molasses Drosophila medium and one drop of 5% live-yeast suspension in water. Larvae placed in the vials were derived from as many sets of parents as possible to ensure maximum genetic diversity in each vial. For example, a vial with an initial $Pgm\text{-}1^{100}$ frequency of 0.25 contained 15 larvae of that genotype (one from each of 15 different parental female lines) and 45 larvae of the $Pgm\text{-}1^{104}$ genotype (two from each of 20 different parental female lines and one more from five of these lines). The 40 experimental vials were then arranged in a 5 × 8 randomized block and placed in an 18° incubator with a 12-hr light/12-hr dark cycle for development.

Adults emerging from the vials were collected daily from the 19th to 34th day after initiation of the experiment. Each day, the emerging flies from each vial were frozen separately for later electrophoresis. In all, 1,244 flies were scored for their *Pgm-1* genotype. The electrophoresis methods used are those of AYALA *et al.* (1972).

RESULTS

The frequencies of the two alleles in the natural Redwood City population are $Pgm\text{-}1^{100} = 0.681$ and $Pgm\text{-}1^{104} = 0.312$, with the remaining 0.007 made up by three rare alleles. The observed frequency of heterozygotes is 0.465 (356 out of 765 females), while the Hardy-Weinberg expected frequency is 0.439 (335.7 of the 765 females).

Frequency dependence was tested with respect to two fitness components, rate of development and larva-to-adult survival. Rate of development was calculated as the weighted mean duration of development, in days, from first instar larvae to adult emergence. Comparisons of the mean developmental rates for the five replicates of a genotype at each frequency with those of the other genotype at the complementary frequency show a general trend with larvae of the less common genotypes developing more rapidly (Table 1). Regressions of rate of development on percent genotypic frequency did not give significant results in mixed culture for either allele. Examination of the data suggests that the lack of significance of the regression is due to high within-group variance. A significant correlation in rate of development between larvae of the two genotypes in a vial ($r = 0.473$, d.f. $= 28$, $P < 0.01$) was noted, which would occur with slight heterogeneity of culture conditions among vials. This could contribute to within-group variance. A Wilcoxon Sign-Rank test for paired comparisons was then conducted to test for differences in rate of development between larvae of the less common and more common genotypes in the 30 (six frequencies × five replicates) mixed-culture vials. This test shows a significant difference in rate of development between the pairs ($P < 0.025$), with larvae of more common genotypes taking significantly longer to develop. A Sign-Rank test for differences between less common and more common genotypes for females only is also

TABLE 1

Mean rate of development in days (with standard error) and regression coefficient of rate of development on percent genotypic frequency

Pgm-1 genotype		Frequency 0.10	0.25	0.40	0.60	0.75	0.90	1.00	Regression r	F(1,28)
100/100	Total	23.30	23.15	23.37	23.45	23.46	23.60	23.18	0.004	1.17
		±0.17	±0.41	±0.18	±0.25	±0.12	±0.41	±0.18	±0.004	
	Females	23.06	22.88	22.92	23.35	23.15	23.35	23.17	0.005	1.29
		±0.26	±0.32	±0.22	±0.39	±0.13	±0.46	±0.14	±0.004	
	Males	23.47	23.31	24.08	23.56	23.76	23.90	23.48	0.007	2.43
		±0.17	±0.69	±0.18	±0.22	±0.10	±0.55	±0.24	±0.004	
104/104	Total	23.27	22.70	23.28	23.97	23.30	23.12	23.62	0.003	0.36
		±0.58	±0.29	±0.31	±0.35	±0.30	±0.27	±0.27	±0.006	
	Females	22.03	22.06	23.40	23.42	23.04	22.69	23.63	0.101	2.88
		±0.40	±0.45	±0.57	±0.32	±0.34	±0.31	±0.36	±0.007	
	Males	24.65	23.68	23.24	24.50	23.60	23.67	23.72	−0.006	0.70
		±0.93	±0.22	±0.19	±0.54	±0.29	±0.24	±0.33	±0.007	

$N = 5$. None of the F values is statistically significant at the five-percent level.

TABLE 2

Mean larva-to-adult survival

Pgm-1 genotype	Frequency 0.10	0.25	0.40	0.60	0.75	0.90	1.00	Regression r	Regression $F(1,28)$	ANOVA for means Mixed culture $F(5,20)$	ANOVA for means Pure and mixed $F(6,24)$
100/100	63.66	61.24	56.06	57.41	60.61	54.32	56.62	−0.082	4.24*	1.88(ns)	1.93(ns)
	±2.22	±3.64	±3.24	±2.86	±2.41	±1.16	±1.00	±0.040			
104/104	64.03	59.42	57.16	56.20	53.57	52.77	56.06	−0.130	6.11*	1.49(ns)	0.89(ns)
	±6.84	±3.84	±3.62	±1.98	±2.97	±1.28	±3.10	±0.053			

Entries give mean arcsin-transformed percent survival, in degrees, with standard errors. Regression coefficient is for arcsin-transformed percent survival on percent genotype frequency. Analysis of variance is for heterogeneity of means for mixed cultures only and for pure and mixed cultures. $N = 5$.

* Significant at $P < 0.05$; (ns) = not significant.

significant ($P = 0.0104$), with less common genotypes developing faster. A similar test for males only is not significant ($P > 0.05$), although males of the less common genotype average a shorter development time than do those of the more common genotype.

The regression of the arcsin-transformed percent survival of each genotype on its input frequency in mixed culture is statistically significant for both the *Pgm-1*^{100} and the *Pgm-1*^{104} genotypes (Table 2). Deviations from linearity of the regressions were nonsignificant. That the regression is negative indicates significant frequency-dependent selection for survival, with less common genotypes having a higher survival rate. An analysis of variance for randomized block design to test for heterogeneity of means gives nonsignificant results for comparison of mixed cultures only, as well as of pure and mixed cultures together. A significant regression without significant heterogeneity of means is often associated with cases where differences among means border on significance (SOKAL and ROHLF 1969). A survey of the means (Table 2) indicates that low and high frequencies of genotypes are associated with high and low survivals, respectively, when compared to pure culture. Larvae in intermediate frequencies (0.40 to 0.60) tend to have survivals equivalent to those in pure culture. In pure culture the genotypes are nearly equivalent to each other in survival ($t_s = 0.173$, d.f. $= 8$, $P > 0.5$). Thus, the interaction between flies of alternative genotypes in the mixed cultures enhances the survival rate of genotypes in low frequencies, while reducing the survival rate of genotypes in high frequencies. This is confirmed by the significant negative correlation that exists for survival between the alternative genotypes in mixed culture vials ($r = -0.475$, d.f. $= 28$, $P < 0.01$).

A replacement series diagram was utilized to assess further the nature of the frequency-dependent selection for survival (AYALA 1971). None of the differences between total observed and expected output were statistically significant, indicating larvae of both genotypes utilize the same resources, with neither cooperative nor disruptive interactions.

The expected equilibrium gene frequency on the assumption that frequency dependence for survival-related components of fitness is the only selective factor present is estimated by regressing the log of the output ratios of genotypes (*Pgm-1*^{104}/*Pgm-1*^{100}) on the log of the input ratios (AYALA 1971). The regression coefficient (0.9347 ± 0.0298) is significantly smaller than one ($t_s = 2.191$, d.f. $= 28$, $P < 0.05$). This indicates a frequency dependence in favor of the low-frequency genotypes, which would lead to a stable equilibrium at the intersection of the regression line with the diagonal with unity slope. The point of intersection corresponds to a frequency of *Pgm-1*$^{100} = 0.615$ for the two-genotype system of this study. It is worth noticing that this expected equilibrium frequency is similar to the observed frequency of the *Pgm-1* allele in the natural population (0.681), although other selective factors appear to influence the equilibrium frequency in natural populations (SNYDER and AYALA, in preparation).

DISCUSSION

The application of the techniques of gel electrophoresis to population genetics (HARRIS 1966; LEWONTIN and HUBBY 1966) revealed a wealth of genetic varia-

tion in natural populations, a discovery that brings about the "paradox of variation" (LEWONTIN 1974). The maintenance of numerous polymorphisms by heterozygote advantage necessitates a "genetic load" that might be greater than finite populations can sustain. The neutrality theory of protein evolution (KIMURA 1968; KING and JUKES 1969) propounds a possible resolution of the paradox—if most allelic variants are adaptively equivalent, there is little or no "genetic load." Frequency-dependent selection is another possible solution—the "load" would be greatly reduced at equilibrium if most polymorphisms are maintained by balancing frequency-dependent selection (KOJIMA 1971).

KOJIMA and YARBROUGH (1967) and KOJIMA and TOBARI (1969) have demonstrated frequency-dependent selection at two allozymic loci in *D. melanogaster*. Frequency-dependent selection has also been demonstrated for inversion polymorphisms (TOBARI and KOJIMA 1967; NASSAR, MUHS and COOK 1973; ANDERSON and WATANABE 1974; GROMKO and RICHMOND 1978; DEBENEDICTIS 1978), male mating success associated with a variety of distinguishing features (EHRMAN 1970), and genotypic strains of cereal grasses (ALLARD and ADAMS 1969). Despite the success of these researchers in detecting frequency-dependent selection and the power of that selective mode in maintaining variation in finite populations (HEDRICK 1972; GROMKO 1977), frequency-dependent selection remains insufficiently explored in experimental population genetics.

It is not possible, of course, to state that the frequency-dependent selection observed in the present study is affecting the *Pgm-1* locus *per se*. Rather, the *Pgm-1* locus might act as a marker for a block of tightly linked genes upon which selection may be operating. The *Pgm-1* locus and genes linked to it are not associated with chromosomal inversions. The only known chromosomal inversion of the *X* chromosome in *D. pseudoobscura* is associated with the sex-ratio trait (DOBZHANSKY and EPLING 1944). The use of 40 independent single-female lines, twenty for each homozygous genotype, makes it unlikely that the experimental results are due to linkage disequilibrium created by the experimental procedure, a problem that may have affected the results of KOJIMA and his colleagues (JONES and YAMAZAKI 1974). The experimental lines used here represent at least 40 different independently derived chromosomes, a number sufficient to contain more than 95% of all the genetic variation present in the natural population. Therefore, if the selection observed does not affect the *Pgm-1* locus *per se*, but reflects a linkage-disequilibrium association of *Pgm-1* with other loci, such disequilibrium is likely to be present in the natural population; hence, the frequency-dependent selection would be affecting (even though indirectly) the *Pgm-1* locus in nature.

Only the two common homozygous genotypes were utilized in this study in order to avoid limiting the data analysis to a few comparisons, as was necessitated by the experimental design of YAMAZAKI (1971). HUANG, SINGH and KOJIMA (1971) noted that allele frequency in "conditioning" genotypes was of primary importance in determining the results of their conditioned medium experiments; thus, the use of only homozygous genotypes may not greatly influence results. Restricting the study to homozygous genotypes also eliminates the

problem of heterosis superimposed on frequency-dependent selection, although no significant heterosis in survival was observed at the *Pgm-1* locus of *D. pseudoobscura* in another study (SNYDER and AYALA, in preparation).

Frequency-dependent developmental rate has not previously been reported in the literature. YAMAZAKI (1971) studied developmental rate in the sex-linked *Est-5* locus of *D. pseudoobscura*, but failed to detect frequency dependence for egg-to-adult or adult-to-adult developmental times. This is not surprising as YAMAZAKI had five genotypes emerging from cultures established at variable densities, used only three frequencies near the observed equilibrium frequency and, presumably, had few replicates (he does not give sample sizes). A correlation between developmental times of various genotypes in a culture, as has been found in our experiments, is expected and does contribute to error variance when an analysis of variance is used to test results. Developmental time of all genotypes in a Drosophila culture normally has low variance; therefore small increases in error variance make analysis of variance a poor test for frequency dependence if genotypes are nearly equal in rate of development. In our study the use of a paired-comparison test demonstrates significant frequency-dependent developmental rates. While the paired-comparison test is appropriate whenever only two genotypes are cultured together, it does not permit quantification of the amount of frequency dependence or treatment of multigenotype cultures. Only large numbers of replicates could reduce error variance sufficiently for detecting frequency-dependent rate of development in multigenotype cultures by analysis of variance (and by regression) unless the selection was very strong.

In this experiment, neither was rate of development significantly reduced in mixed cultures relative to pure cultures (Table 1) nor was overall facilitation of survival in genotypically mixed cultures found. MARINKOVIC and AYALA (1975) reported both a reduction in rate of development and a facilitation of survival under marginal temperature and high-density conditions using flies that varied at both the *Me-2* and *Pgm-1* loci. Facilitation of survival has also been reported for *D. pseudoobscura* in cultures heterogeneous for chromosomal inversions (BEARDMORE 1963). The current study differs from that of MARINKOVIC and AYALA by the use of larger numbers of single-female lines, which may have resulted in an overall increase of diversity within pure cultures.

Significant frequency dependence in larva-to-adult survival occurs for both *Pgm-1* genotypes. The nature of the interaction between the genotypes involves the reduction of survival rate in the more common genotype and its enhancement in the less common genotype. When both genotypes occur at intermediate, near-equilibrium frequencies, the two genotypes exhibit nearly identical survival rates, as predicted by the frequency-dependent selection model of KOJIMA and YARBROUGH (1967). The predicted equilibrium frequencies at the locus, based solely on frequency-dependent effects for survival-related components of fitness, in this experiment are $Pgm\text{-}1^{100} = 0.615$ and $Pgm\text{-}1^{104} = 0.385$. These frequencies are not greatly different from those in the natural population at the time when the wild flies were collected. In a two-year period, the frequency of the $Pgm\text{-}1^{100}$ allele varied from a low of 0.55 to a high of 0.71 in the MacDonald

Ranch population of *D. pseudoobscura* monitored by DOBZHANSKY and AYALA (1973). The frequency-dependent selection observed in this experiment could maintain a polymorphism within this frequency range if it were the only selective force acting on the population and the addition of the heterozygous genotype did not influence the equilibrium. Differences among genotypes for fertility components of fitness could influence the equilibrium frequency, as could the addition of the heterozygous genotype. However, SNYDER and AYALA (in preparation) did not detect any heterogeneity among genotypes at this locus for age-specific female fertility or more mating capacity.

Frequency-dependent selection may be an important mechanism for maintaining balanced polymorphisms in natural populations. A balanced polymorphism at the *Pgm-1* locus of *D. pseudoobscura* could be maintained adequately by frequency dependence of larva-to-adult survival and of rate of development. However, other results (SNYDER and AYALA, in preparation) suggest that frequency dependence may act together with other selective factors responding to variable temperature and density, which may account for seasonal oscillations in gene frequencies at the *Pgm-1* locus in natural populations (DOBZHANSKY and AYALA 1973). Although studies to date have often found evidence for frequency-dependent selection when properly designed, much further work is needed before we can adequately understand the role and extent of this selective mode in maintaining balanced polymorphisms in natural populations.

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Corresponding editor: W. W. ANDERSON