FITNESS OF ALLOZYME VARIANTS IN *DROSOPHILA*
PSEUDOOBSCURA. I. SELECTION AT THE *PGM-1* AND *ME-2* LOCI¹

DRAGOSLAV MARINKOVIĆ AND FRANCISCO J. AYALA

*Faculty of Science, University of Belgrade, Belgrade, Yugoslavia, and Department of Genetics,
University of California, Davis, California 95616*

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ABSTRACT

We have studied in *Drosophila pseudoobscura* the effect of allozyme variation on seven fitness components: female fecundity, egg hatchability, egg-to-adult survival under near-optimal and under competitive conditions, rate of development under near-optimal and under competitive conditions, and mating capacity of males. Three genotypes at each of two loci, *Pgm-1* and *Me-2*, have been studied in various combinations. These two loci are highly polymorphic in natural populations of *D. pseudoobscura*. Statistically significant differences involving one or more genotypes exist for all components of fitness. No single genotype is best for all fitness components; rather the relative fitnesses of genotypes are reversed when different parameters are considered, or when they are studied in different environmental conditions. Also, the average egg-to-adult survival and rate of development are better when different genotypes are reared together than when they occur in pure culture. Four different modes of selection have been uncovered by our experiments. These forms of selection may account for the persistence of the two allozyme polymorphisms in nature, and for previously observed seasonal fluctuations of the allelic frequencies in natural populations.

ELECTROPHORETIC studies of genetic variation in natural populations of different species of *Drosophila* are now quite numerous (for a review of the literature up to 1972, see LEWONTIN 1974). A great deal of allelic variability has been found, and many authors have presented evidence indicating that much of the variation is maintained by balancing natural selection (e.g., PRAKASH and LEWONTIN 1968, 1971; AYALA, POWELL and TRACEY 1972; AYALA *et al.* 1972, 1974; RICHMOND 1972; ZOUROS and KRIMBAS 1973; DOBZHANSKY and AYALA 1973).

A number of laboratory studies have been directed toward ascertaining the mode of selection involved in maintaining allozyme polymorphisms. Changes in allelic frequencies due to natural selection have been demonstrated at the *Mdh-2* locus in *D. willistoni*, *D. equinoxialis*, and *D. tropicalis* (AYALA and ANDERSON 1973). In *D. willistoni* there is also evidence of selection at the *Lap-5* and *Est-5* loci, but not at the sex-linked, *Est-7* (POWELL 1973). YAMAZAKI (1971)

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Reprint requests from Europe should be addressed to D. MARINKOVIĆ; from elsewhere, to F. J. AYALA.

found no evidence, either, of natural selection at the sex-linked esterase locus, *Est-5*, in *D. pseudoobscura*, although FONTDEVILA (1973 and personal communication) has recently found evidence of selection at the locus. The difference between these two reports may be due to the fact that YAMAZAKI's study was started with strains of flies kept in the laboratory for many years before the beginning of the experiment, while FONTDEVILA's experimental populations were started with the descendants of flies recently collected in nature. MACINTYRE and WRIGHT (1966) obtained inconclusive results at a sex-linked esterase locus, *Est-6*, in both *D. melanogaster* and *D. simulans*. RASMUSON, RASMUSON and NILSON (1967) studied experimental populations of *D. melanogaster* with different initial frequencies of the *Est-6^F* and *Est-6^S* alleles; the authors considered it probable that the *Est-6* polymorphism was maintained by balancing selection affected by the genetic background. In *D. virilis*, OHBA (1969) found no significant frequency changes at two loci, *Est-2* and *Est-7*, although changes occurred at the *Est-9* locus.

There are as yet very few studies attempting to ascertain what fitness components are affected by the electrophoretic variants. KOJIMA and YARBROUGH (1967) and KOJIMA and TOBARI (1969) have reported that genotypic fitnesses for viability are frequency-dependent at the *Est-6* and *Adh* loci in *D. melanogaster*. On the other hand, YAMAZAKI (1971) found no significant differences in several fitness components among *Est-5* genotypes of *D. pseudoobscura*.

The experiments reported here were made to ascertain whether fitness components in *D. pseudoobscura* are affected by genotypic variation at two loci, *Pgm-1* and *Me-2*. DOBZHANSKY and AYALA (1973) have shown that allelic frequencies at these two loci fluctuate through the seasons in two natural populations, and that the fluctuations are repeated from one year to the next. The oscillations observed by DOBZHANSKY and AYALA might conceivably be due to differential selective values at different stages of the life cycle, perhaps associated with changes in population density or with environmental parameters. At each of the two loci studied by DOBZHANSKY and AYALA, only two alleles occur in substantial frequencies, and together have a frequency of about 95% or higher. Our studies are concerned with these two common alleles at the *Pgm-1* and *Me-2* loci.

MATERIALS AND METHODS

The *Pgm-1* locus is sex-linked while *Me-2* is in the second chromosome. These loci code for the enzymes, phosphoglucumutase-1 and malic enzyme-2, respectively. Our techniques of starch gel electrophoresis and the enzyme assays used have been described elsewhere (AYALA, POWELL and TRACY 1972).

We have used several strains descended from flies collected in the fall of 1972 at MacDonald Ranch, Napa County, California. Several thousand inseminated wild females were placed in individual cultures. Single-pair sib matings were made with the F_1 progenies to establish the experimental strains. The genotypes of the two parents were ascertained by electrophoresis, and strains having the desired genetic constitutions were chosen. In some cases, a second generation of sib pair matings was obtained. There are three groups of strains. The first one (group A) consists of 10 strains, each homozygous for *Pgm-1¹⁰⁴* and for *Me-2¹⁰⁰*. The second one (B) consists of 6 strains homozygous for *Pgm-1¹⁰⁰* and *Me-2¹⁰²*. The third group (C) consists of 12 strains with flies homozygous for *Pgm-1¹⁰⁰* and *Me-2¹⁰⁰*. The number of strains simultaneously

homozygous for the two rare alleles (*Pgm-1*¹⁰⁴ and *Me-2*¹⁰²) was too small, and we decided not to use them. Each one of our strains descends from a different wild inseminated female. At the beginning of our experiment, the flies were fourth or fifth generation descendants of the wild flies.

To randomize the genotypes, series of crosses between the strains of each group have been performed over two generations. If the strains of group A are represented as A₁, A₂, A₃, etc., we have first crossed A₁ × A₂, A₂ × A₃, etc.; then, we have crossed A₁₋₂ × A₃₋₄, A₂₋₃ × A₄₋₅, etc. Thus, we have obtained within each group five combined strains, each originating from four different ancestral strains. In the third generation, crosses have been made between pairs of such synthetic strains within the same group (e.g., A₁₋₄ × A₅₋₈; or B₃₋₆ × B₁₋₂; or C₂₋₅ × C₆₋₉, etc.), and also between strains from different groups (e.g., A₁₋₄ × B₁₋₄; B₂₋₅ × C₂₋₅; or A₃₋₆ × C₃₋₆, etc.). Consequently, six different groups, each with five synthetic strains, have been obtained. Each of these synthetic strains is derived from at least six (B), and generally eight, original strains. All synthetic strains should have much of the genetic variation in the original population, except for the two loci under consideration. The genotypes of the synthetic strains at the *Pgm-1* and *Me-2* loci are as follows:

	A	B	C	A × B	A × C	B × C
<i>Pgm-1</i>	104/104	100/100	100/100	104/100	104/100	100/100
<i>Me-2</i>	100/100	102/102	100/100	100/102	100/100	102/100

The 30 synthetic strains (five in each of the six groups) have been raised simultaneously, and hatchability of their eggs, egg-to-adult survival, and rate of development have been measured under near-optimal conditions (i.e., 100 eggs per half-pint bottle, with two replications per strain). Also, egg-to-adult survival and rate of development have been measured under competitive conditions (i.e., 200 eggs per 11 mm-diameter vial, with one replica per strain). The females raised under optimal conditions have been compared for fecundity, by measuring the number of eggs laid by each of two groups of 3 females with 3 males from each strain, from the 6th to the 13th day after emergence (see MARINKOVIĆ 1967). Males, raised under optimal conditions, were compared for their mating ability; males from each strain were placed individually in a bottle with 8 virgin females taken from a common pool of all 30 synthetic strains. Number of females inseminated by each male after 24 hours was determined by inspecting the reproductive tracts of the females for presence of sperm.

Egg-to-adult survival and rate of development have also been measured in two sets of vials, each vial containing 198 eggs. In the first set, the eggs in a vial all came from the five synthetic strains of a given group. In the second set, 33 eggs came from each of the six groups of strains.

All cultures were kept in a constant temperature incubator at 25 ± 0.5° and ca. 70% relative humidity. The food was standard agar-cornmeal-molasses *Drosophila* medium.

The following should be noted concerning the statistical methods. We are studying three genotypes at each of two loci; nine different genotypes are possible when both loci are considered simultaneously. Owing to the difficulty of obtaining enough strains homozygous simultaneously for the two rare alleles, we have used in our experiment only six of the nine possible genotypes. Therefore, it is not possible to carry out a straightforward two-way analysis of variance to separate the effects of the two loci and to detect interactions between them. Instead, we have performed a series of nonorthogonal linear contrasts for each of the two most common genotypes at each locus, and for the interaction between the two loci. This procedure was advised by a reviewer of a previous version of this paper.

For each fitness component we have tested five hypotheses, namely whether each of *Pgm-1*^{100/100}, *Pgm-1*^{100/104}, *Me-2*^{100/100}, *Me-2*^{100/102}, and the interaction, cause a significant deviation from the mean effect of all the genotypes. The following matrix of coefficients was used for the nonorthogonal comparisons. (See above for the genotypes of each group of strains.)

Genotype tested	Strains					
	A	B	C	A × B	A × C	B × C
<i>Pgm-1</i> ^{100/100}	—4	0	7	—5	—3	5
<i>Pgm-1</i> ^{100/104}	—4	0	—5	7	9	—7
<i>Me-2</i> ^{100/100}	0	—4	7	—5	5	—3
<i>Me-2</i> ^{100/102}	0	—4	—5	7	—7	9
Interaction	0	0	—6	—6	6	6

The degrees of freedom for each contrast are 1, $6(n-1)$, where n is the number of replications made in the study of a given fitness component. In the few cases where a replicate was missing for a given genotype, we have replaced it by the mean value of the other replicates, and reduced accordingly the degrees of freedom of the denominator.

RESULTS

Table 1 gives the means and standard errors of the fitness components for each of the six groups of strains. The number of replications is 5, 10, or 20, depending on the experiment. Each group of strains has the specified genotype at the *Pgm-1* and *Me-2* loci. In three groups (A, B, C) flies are homozygous at both loci; in two groups (A × C and B × C) flies are homozygous at one locus and heterozygous at the other; in one group (A × B) flies are heterozygous at both loci. Overall statistical significance of the differences among the six groups is tested by one-way analyses of variance.

Differences in fitness components are statistically significant in four fitness parameters: female fecundity, hatchability, egg-to-adult survival under competitive conditions, and mating capacity of males. The analysis of variance indicates no significant differences in egg-to-adult survival under near-optimal conditions, nor in rate of development.

Flies heterozygous at one or both loci are ostensibly superior to flies homozygous at both loci in fecundity (average, 190 ± 10 versus 153 ± 13 eggs per female per 7 days) and in male mating capacity (4.66 ± 0.24 versus 3.56 ± 0.28 females insiminated per male per 24 hours). However, the homozygotes have better egg-to-adult survival, although not significantly so (92.2 ± 1.8 versus $85.8 \pm 3.3\%$, and 44.8 ± 3.2 versus $38.5 \pm 3.0\%$, under optimal and competitive conditions, respectively).

Among the homozygotes, the carriers of the two alleles most common in the natural population (*Pgm-1*¹⁰⁰ and *Me-2*¹⁰⁰) have the highest fecundity, but the lowest male mating capacity. Flies homozygous for the most common *Pgm-1* allele (100) and the less common *Me-2* allele (102) have the lowest fecundity, but the highest hatchability and male mating capacity.

To dissociate the fitness effects of the two loci we have made analyses of variance for single degree of freedom contrasts between each of the two more common genotypes at each locus and all other genotypes. The results are summarized in Table 2. At either locus, homozygotes for the rare allele are not shown in this table. This is because the rare homozygotes occur only in one combination, namely associated with the most common homozygote at the other locus. Therefore there is no way to disassociate the fitness contribution of the rare homozygotes from their interaction with the genotype at the other locus. The hypothesis being tested in Table 2 is whether the contribution to fitness of each genotype

TABLE 1
Means and standard errors for seven fitness parameters in six genetically different strains of *Drosophila pseudoobscura*

N is the number of replications.

Genotype	<i>Pgm-1</i> † <i>Me-2</i>	Strains						N	Degrees of freedom	F
		A 104/104 100/100	B 100/100 102/102	C 100/100 100/100	AxB 104/100 100/102	AxC 104/100 100/100	BxC 100/100 102/100			
Female fecundity‡		146 ± 16	100 ± 18	213 ± 16	174 ± 15	177 ± 22	220 ± 10	10	5,54	7.24***
Percent egg hatchability		76.7 ± 4.7	93.2 ± 1.7	87.1 ± 2.6	86.0 ± 4.6	87.5 ± 2.7	91.5 ± 2.9	5	5,24	2.89*
Percent egg-to-adult survival:										
optimal conditions		93.9 ± 1.8	96.0 ± 1.3	86.7 ± 4.7	87.4 ± 5.2	90.0 ± 3.4	79.9 ± 7.7	10	5,53	1.63
competitive conditions		50.7 ± 1.9	40.9 ± 3.8	42.6 ± 8.8	35.7 ± 2.9	50.4 ± 3.0	29.4 ± 4.4	5	5,23	2.96*
Rate of development (in days):										
optimal conditions		13.50 ± .14	13.75 ± .13	13.32 ± .14	13.55 ± .22	13.20 ± .16	13.24 ± .24	10	5,54	1.19
competitive conditions		22.3 ± 1.0	21.2 ± 0.8	22.4 ± 0.7	23.0 ± 1.0	21.3 ± 0.2	19.6 ± 1.5	5	5,23	1.51
Mating capacity of males‡		3.75 ± .49	4.59 ± .32	2.95 ± .57	4.50 ± .42	5.01 ± .48	4.48 ± .38	20	5,109	3.56**

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

† The genotype of males at the *Pgm-1* locus is 100/Y in strains B, C, and BxC; and 104/Y in strains A, AxB, and AxC.

‡ Female fecundity is given as the number of eggs laid by a female for seven days; the mating capacity of males is the number of females inseminated per male in 24 hours.

TABLE 2

Effect of genotype at two enzyme loci in each of seven fitness parameters

The deviation from the mean due to each genotype is given above; below, *F* statistic for the significance of the deviation.

	Pgm		Me		Interaction	Degrees of freedom
	100/100	100/104	100/100	100/102		
Female fecundity	+34.0 101.9***	-11.9 16.6***	+18.6 30.2***	-12.0 16.8***	+2.3 0.47	— 1, 54
Percent egg hatchability	+2.12 14.9***	+0.19 0.09	-1.28 3.04	-0.16 0.06	+1.49 4.11	— 1, 24
Percent egg-to-adult survival:						
optimal conditions	+0.57 0.39	+1.67 4.44*	-0.13 0.01	-3.64 21.2***	-1.05 1.32	— 1, 53
competitive conditions	-3.64 7.02*	+2.56 10.5**	+5.00 30.17***	-1.40 3.14	+0.35 0.07	— 1, 23
Rate of development (in days):						
optimal conditions	-0.08 5.06*	+0.01 0.14	-0.13 14.5***	0.00 0.00	-0.11 9.24***	— 1, 53
competitive conditions	-0.54 7.14**	+0.44 6.40*	+0.16 2.16	-0.26 2.31	-1.13 31.9***	— 1, 23
Mating capacity of males	-0.35† 11.1***	+0.66‡ 51.8***	-0.40 14.5**	+0.11 1.39	+0.56 28.7***	— 1, 109

* Statistical significance: $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† The male genotype is 100/Y.

‡ The male genotype is 104/Y.

is significantly different from the average effect of all genotypes. The table gives the deviation from the mean due to each genotype, and the corresponding *F* value. The magnitude and *F* value of the interaction between the two loci are also given.

Significant interactions between the two loci occur for mating capacity of the males and for rate of development both under optimal and under competitive conditions. For egg hatchability, the interaction is at the edge of the conventional level of significance ($F = 4.11$, $F_{(0.05)} = 4.26$). One or more genotypes have statistically significant effects for every one of the seven fitness parameters studied. All four genotypes have significant effects on female fecundity, and three of them affect the mating capacity of males. This suggests that genotypic differences may often have greater effect on fitness components associated with fertility than in those associated with viability (see SVED and AYALA 1970; TRACEY and AYALA 1974).

Table 2 shows that alternative genotypes may have opposite effects on different fitness components. At the *Pgm-1* locus, homozygotes for the most common allele (100/100) are significantly superior to the heterozygotes (100/104) in female fecundity, but the heterozygotes are superior in egg-to-adult survival under competitive conditions, and in rate of development under both optimal and competitive conditions. At the *Me-2* locus the homozygotes (100/100) are superior to the heterozygotes (100/102) in female fecundity, but the heterozygotes

TABLE 3

Means and standard errors for average egg-to-adult survival and rate of development under competitive conditions for six genetically different strains of Drosophila pseudoobscura
N is the number of replications.

	<i>N</i>	Percent survival	Rate of development (in days)
Pure cultures	30	41.3 $\pm$ 2.3	21.64 $\pm$.43
Mixed cultures	8	50.1 $\pm$ 2.0	16.49 $\pm$.27

are superior in rate of development under competitive conditions and in mating capacity of males.

To examine the ability of a population of mixed genotypes to exploit a limited environment we have studied, under competitive conditions, survival from egg-to-adult and rate of development, in pure and in mixed cultures. One hundred and ninety-eight eggs were placed in a vial with food. In pure cultures all eggs within a vial were genetically identical at the *Pgm-1* and *Me-2* loci; all six groups of strains were tested with five replicates for each group. In mixed cultures, 33 eggs from each of the six groups of strains were placed together in a vial (eight replications). The results are summarized in Table 3. In pure cultures, the average survival of all six kinds of genotypes is $41.3 \pm 2.3\%$ and the average rate of development is 21.64 ± 0.43 days. Average performance is higher in the mixed cultures for both survival ($50.1 \pm 2.0\%$, $t = 2.89$, $p < 0.05$) and rate of development (16.49 ± 0.27 days, $t = 10.10$, $p < 0.001$). The average egg-to-adult survival in mixed cultures is about the same as that of the best genotype in pure culture ($50.7 \pm 1.9\%$ for *Pgm-1*^{104/104}, *Me-2*^{100/100}; see Table 1), while the rate of development is better in mixed cultures than that of the best genotype in pure culture (19.6 ± 1.52 days for *Pgm-1*^{100/100}, *Me-2*^{100/102}; see Table 1). A population of mixed genotypes makes better use of the environmental resources than a population of identical genotypes.

DISCUSSION

Natural populations of *Drosophila* as well as of other outcrossing, sexually reproducing organisms have abundant stores of genetic variation. Using techniques of gel electrophoresis and selective assay of enzymes, an individual appears to be heterozygous, on the average, at 15.1% of its loci for 24 invertebrate species, and at 5.8% of its loci for 22 vertebrate species (SELANDER and KAUFMAN 1973). The total amount of genetic variation in natural populations may be much greater than these figures indicate, since only a fraction of all allelic variation is detected by gel electrophoresis.

It has been suggested that most or all allozyme variation may be effectively neutral to natural selection (see, e.g., KIMURA and OHTA 1971). Our results show that, on the contrary, various components of fitness are affected by electrophoretically detectable allelic variants. Six different genotypes at two loci, *Pgm-1* and *Me-2*, have been examined in *D. pseudoobscura*. Our results show that statistic-

ally significant heterogeneity exists among the genotypes in four fitness parameters out of seven examined. The overall variation is significant for female fecundity, hatchability of eggs, egg-to-adult survival under competitive conditions, and male mating capacity (Table 1). When the genotypic effects are studied at each locus separately, it is found that significant deviations from the mean are caused by one or more genotypes in each of the seven fitness parameters (Table 2).

The genetic background of the experimental flies was randomized by multiple crosses among the 28 original strains, each of which was derived from an inseminated female collected in nature. Differences in fitness components among the genotypes are, therefore, presumably due to the two loci under consideration. It is of course possible, and indeed likely, that alleles at the two loci studied exist in nature in linkage disequilibrium with alleles at other loci. Such linkage disequilibrium would remain after the randomizing crosses between the original strains. Our experiments would, in that case, measure the effects of genotype at the *Pgm-1* and *Me-2* loci, in the genetic combinations *that exist in the natural population*. Significant interactions between the *Pgm-1* and *Me-2* loci occur in our experiments for three fitness parameters: rate of development in optimal and in competitive conditions, and male mating capacity (Table 2). Significant interactions with other loci are also likely.

The main purpose of our experiment was to ascertain not only whether natural selection is involved, but also what fitness components might be affected, and whether environmental variation might affect relative fitness. We have shown that a variety of fitness components are affected by allozyme variation, and that the fitnesses depend on the environmental conditions. Several forms of natural selection occur in our experiment.

First, the relative fitnesses of genotypes may be reversed when different fitness components are considered. No single genotype has the highest fitness for all fitness components. This is apparent in Tables 1 and 2. For example, double homozygotes for the most common allele (*Pgm-1*^{100/100} and *Me-2*^{100/100}) have more than twice the fecundity of the double homozygotes, *Pgm-1*^{100/100} and *Me-2*^{102/102}; yet the male mating capacity of the latter is much greater than that of the former (Table 1; notice that the *Pgm-1* genotype is the same in both cases). As pointed out above the *Pgm-1*^{100/100} homozygotes are superior to the *Pgm-1*^{100/104} heterozygotes in female fecundity, but inferior to them in egg-to-adult survival under competitive conditions and in rate of development (see Table 2). Additional examples can be seen in the tables.

KOJIMA and TOBARI (1969) found differences in larval viabilities among genotypes at the *Adh* locus in *D. melanogaster*. They suggested that such "viability differences may be counterbalanced with some other fitness component differences." The net result could be nearly identical fitnesses of all genotypes at equilibrium. Our study gives ample evidence of such "reversals" of relative fitness advantages. It should be pointed out, however, that reversals of fitness advantages at different stages of the life cycle do not by themselves lead to stable equilibrium. If the reversals counterbalance each other exactly so that the net

TABLE 4

Number of adults emerging and mating capacity of males for six different genotypes of Drosophila pseudoobscura

The number of emerging adults is estimated by multiplying the number of eggs laid by one female in 7 days by the survival rate from egg-to-adult.

	Number of emerging adults		Mating capacity of males
	Optimal conditions	Competitive conditions	
<i>Me-2</i> ^{100/100}	161	85	3.7
<i>Me-2</i> ^{100/102}	165	64	4.5
<i>Me-2</i> ^{102/102}	96	41	4.6
<i>Pgm-1</i> ^{100/100}	155	67	4.1
<i>Pgm-1</i> ^{100/104}	156	74	*
<i>Pgm-1</i> ^{104/104}	137	74	4.1

* No male heterozygous for both alleles exists at the *Pgm-1* locus, since it is sex-linked.

fitnesses of the genotypes are identical, only a neutral equilibrium may result. Fitness components reversals may lead to stable equilibrium if their net result is heterosis or if they are frequency-dependent.

The second mode of selection present in our experiment is heterosis of the type which could be called "marginal overdominance" (WALLACE 1968). Table 4 gives the product of fecundity (number of eggs laid per female per 7 days) times percent survival from egg-to-adult, for each genotype at each locus. This product estimates the number of adults emerging per female under the experimental conditions. Under near-optimal conditions, the number of emerging adults is greater for the homozygotes most common in nature than for the rarer homozygotes. This is true at both loci. The heterozygotes produce about the same number of adults as the best homozygote. At the *Me-2* locus the mating capacity of males is greater for the rare than for the common homozygotes, while again the heterozygotes are approximately equal to the best homozygotes, which in this case are those carrying the rarer allele (104). This provides a case of marginal overdominance, because no heterosis exists for either one of the two fitness parameters independently, but the heterozygote is superior to either homozygote when both parameters (number of emerging adults under optimal conditions and mating capacity of males) are considered simultaneously.

Heterosis as well as reversals of fitness components may occur in a spatially uniform and temporally constant, ideal environment. Some selective differences in our experiment are due to environmental heterogeneity. The third mode of selection arises from the spatial heterogeneity of the environment, and it may account for frequency-dependent selection in a variety of situations. The average survival and rate of development are better in mixed cultures, *i.e.*, when several genotypes coexist together, than in pure cultures (see Table 3). Presumably, the mixed genotypes exploit better the environmental heterogeneity. This results in greater carrying capacity of the environment, and also suggests why selection

might be frequency-dependent (AYALA and CAMPBELL 1974). As the frequency of a genotype increases, competition for the environmental resources that the genotype exploits optimally becomes more intense, and the relative fitness of the genotype may decrease. An inverse relationship between the frequency of a genotype and its fitness may lead to stable polymorphic equilibria. KOJIMA and YARBROUGH (1967), and KOJIMA and TOBARI (1969) have demonstrated frequency-dependent selection at two allozyme loci in *D. melanogaster*.

The fourth form of selection is due to changes in the environmental conditions that may lead to changes in relative fitness. We have found that relative fitnesses under optimal conditions are sometimes reversed under competitive conditions (e.g., between *Pgm-1*^{100/100} and *Pgm-1*^{104/104} in Table 4). DOBZHANSKY and AYALA (1973) found cyclical changes associated with the seasons in the allelic frequencies at the *Pgm-1* and *Me-2* loci in two natural populations of *D. pseudoobscura*. Population density, besides temperature, rainfall, and other environmental parameters, change with the seasons. Some genotypes may have a selective advantage in the early spring when the density of the population is low, but be at a selective disadvantage in the summer when the population density is high.

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Corresponding editor: T. PROUT