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TEMPERATURE AND DENSITY EFFECTS ON FITNESS AT THE MDH-2 AND PGM-1 LOCI OF *DROSOPHILA PSEUDOOBSCURA*

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Three fitness components (egg-to-adult viability, female fecundity, and male mating capacity) are studied in *Drosophila pseudoobscura* under four environmental conditions (optimal or marginal temperature; optimal or competitive density). The experimental flies differ in their genotypes at two gene loci coding for enzymes: the sex-linked *Pgm-1* and the fourth-chromosome *Mdh-2*. The *Pgm-1* locus is highly polymorphic in natural populations (heterozygosity approaching 50%); the *Mdh-2* locus moderately so (heterozygosity around 10%). Two alleles, most common in nature, are studied at each locus.

A sample of 1,140 wild females was used to extract lines with the desired genotypes. A total of 80 independent lines homozygous at both the *Pgm-1* and *Mdh-2* loci were obtained, 20 lines for each of the four double homozygotes; heterozygotes at one or both loci were obtained by intercrossing lines homozygous for different alleles.

Genotype at the *Pgm-1* locus has significant fitness effects for egg-to-adult survival under all conditions tested; at the *Mdh-2* locus the effects are significant under competitive conditions at either temperature, but not under optimal density. At both loci the fitness effects are significantly different when the environmental conditions are different. Significant differences exist for fecundity among the *Mdh-2* genotypes at the marginal but not at the optimal temperature; none exist among the *Pgm-1* genotypes at either temperature. No significant genotypic differences exist for male mating capacity. The demonstration of fitness differences among genotypes and of fitness

shifts associated with changing environmental conditions suggests that selection plays a significant role in maintaining variability at the *Pgm-1* and *Mdh-2* loci in *D. pseudoobscura*.

Introduction

Electrophoretic studies have established that natural populations carry considerable amounts of genetic variation. The mechanisms by which such variation is maintained and, in particular, the role played by various types of natural selection is an important question. Some studies have investigated the subject by comparing data from electrophoretic surveys with environmental and ecological parameters or with theoretical constructs in an effort to ascertain whether the observed genetic variation is maintained by selection or is adaptively neutral. Relatively few studies have approached the problem by examining the fitnesses of organisms with alternative genotypes at specific loci coding for enzymes and other proteins (Kojima & Yarbrough 1967; Kojima & Tobari 1969; Marinković & Ayala 1975a, b, 1977; Yamazaki 1971).

The present study examines the effects of temperature and density on three components of fitness for genotypes at the *Mdh-2* and *Pgm-1* loci of *Drosophila pseudoobscura*. The components of fitness studied are egg-to-adult survival, female fecundity, and male mating capacity. These are critical fitness components although they do not include all the life-cycle parameters that might be subject to selection (Prout 1965). The possible effects of environmental variation on fitness are examined with respect to two environmental variables — temperature and density.

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Material and methods

The strains of *Drosophila pseudoobscura* used in the experiment are derived from 1,140 wild females collected in Redwood City, San Mateo County, California, in early September 1976. Single-pair sib matings were made with the F_1 progenies of the inseminated wild females. The F_1 pairs were allowed to oviposit and were then subjected to electrophoresis according to Ayala et al. (1972) to ascertain their genotypes at two gene loci coding for enzymes, *Pgm-1* (X chromosome) and *Mdh-2* (4th chromosome). Lines in which both parents were homozygous (or hemizygous in the case of males at the *Pgm-1* locus) at both loci were maintained separately in mass culture. Due to the low frequency of the *Mdh-2*¹¹² allele in the natural population, four generations of sib-pair matings were required to isolate the lines homozygous for this allele. The same number of sib-pair matings were, however, also made with the other lines in order to ensure a similar average degree of inbreeding in all lines. These crosses were all done at 18°C. In all, 20 independent lines were obtained for each of four doubly-homozygous genotypes as follows:

Lines A₁ to A₂₀: *Pgm-1*^{104/104} and *Mdh-2*^{112/112}

Lines B₁to B₂₀: *Pgm-1*^{100/100} and *Mdh-2*^{112/112}

Lines C₁to C₂₀: *Pgm-1*^{104/104} and *Mdh-2*^{100/100}

Lines D₁to D₂₀: *Pgm-2*^{100/100} and *Mdh-2*^{100/100}

By intercrossing lines from different sets, 20 lines were obtained for each of the five other desired genotypes (four heterozygous at one or the other locus and one heterozygous at both loci), as follows:

Lines AB₁to AB₂₀: *Pgm-1*^{104/100} and *Mdh-2*^{112/112}

Lines AC₁to AC₂₀: *Pgm-1*^{104/104} and *Mdh-2*^{112/100}

Lines BC₁to BC₂₀: *Pgm-1*^{104/100} and *Mdh-2*^{112/100}

Lines BD₁to BD₂₀: *Pgm-1*^{100/100} and *Mdh-2*^{112/100}

Lines CD₁to CD₂₀: *Pgm-1*^{104/100} and *Mdh-2*^{100/100}

In order to avoid the effects of inbreeding, the eggs used to study egg-to-adult survival always came from crosses between two different lines (e.g., ♀A₁ × ♂A₂, ♀A₂ × ♂A₃, etc., for the genotype *Pgm-1*^{104/104}, *Mdh-2*^{112/112}, and ♀A₁ × ♂B₁, ♀A₂

× ♂B₂, etc., for the genotype *Pgm-1*^{104/100} and *Mdh-2*^{112/112}). These eggs carry two independent genomes from the natural population and thus have genetic constitutions similar to those of natural flies with the desired genotype at the loci under study. All fitness components were studied at two temperatures, 18° and 26°C. For *D. pseudoobscura*, 18°C is an optimal temperature while 26°C is marginal and near the upper limit at which the species can successfully reproduce. Near-optimal (or, simply, optimal) density was defined as 20 eggs per 18 mm.-diameter vial with 10 ml of cornmeal-agar-molasses *Drosophila* medium; competitive density was defined as 200 eggs per 18 mm.-diameter vial. Each optimal density vial contained four eggs from each of five lines (A₁₋₅, A₆₋₁₀, CD₁₅₋₂₀, etc.), a design arbitrarily chosen to facilitate the large amount of egg handling required. A total of 20 replicate vials were set up for each genotype at each temperature. The competitive density vials were similarly set up, but only eight replicates were established for each genotype at each temperature. The experiments were conducted similarly at both temperatures, except that replicates of the original lines were allowed to develop for one generation at 26°C before starting the experiment at this temperature. The eggs initiating the experiments at 26°C were thus one generation removed from those used for the 18°C experiments. At both temperatures, flies emerging from the vials were collected daily and counted by sex. Those from optimal density vials were saved for the fecundity and male mating capacity experiments.

For the fecundity experiments, seven-days-old virgin females that had never been etherized were placed in groups of three with three males coming from a common pool. The number of eggs laid on plastic spoons containing standard medium by each group of three females was counted daily for eight consecutive days starting one day after the females and males were placed together. (Thus the fecundity values represent the numbers of eggs laid by three females from the eighth to the fifteenth day of their lives. *D. pseudoobscura* females rarely lay eggs before the fifth day of adult life (Marinković 1967).) Twelve replicate vials were set up for each genotype at each temperature.

Results

The allelic frequencies at the *Pgm-1* and *Mdh-2* loci in the Redwood City population of *Drosophila pseudoobscura*, based on a sample of 1,530 wild genomes, are as follows:

Pgm-1 100 = 0.681, 104 = 0.312, other = 0.007

Mdh-2 100 = 0.953, 112 = 0.032, other = 0.015

Thus the two alleles used at each locus in our study are the most common ones in the natural population from which the flies originate. (The 'other' alleles include three rare ones in each case.) The *Pgm-1* locus is highly polymorphic, with an observed frequency of heterozygotes of 0.465 (356 out of 765 females; Hardy-Weinberg expected heterozygosity, 0.439 or 335.7 females). The *Mdh-2* locus is moderately poly-

morphic, with an observed frequency of heterozygotes of 0.102 (78 out of 765 females; expected, 0.091 or 69.3 females).

The results of the egg-to-adult survival in optimal density are summarized in Table 1. As expected, the rate of survival is greater for every genotype at the optimal 18°C temperature (the average survival rate for all nine genotypes is 70.9%) than at the marginal 26°C temperature (average survival rate, 28.7%).

More important from the present point of view are the significant differences among the *Pgm-1* genotypes with respect to egg-to-adult survival. These differences and the relevant tests are shown in Table 2. At 18°C flies of the *Pgm-1*^{104/104} genotype have a higher survival rate than either the heterozygous or the *Pgm-1*^{100/100} homozygous flies. This is true for total progeny as well as for females and males analyzed separately. The same trend of higher survival of

Table 1

Egg-to-adult survival under optimal density: mean and standard error of the number of adults emerging from 20 eggs having the given genotype. The mean numbers of emerging females and of males are also given. The number of replicates is 20.

MDH genotype	Group	104/104	PGM genotype 104/100*	100/100
112/112	18 ° Total	16.25 ± 1.00	15.40 ± 0.72	12.00 ± 1.03
	18 ° ♀♀	8.40 ± 0.66	7.35 ± 0.65	6.25 ± 0.78
	18 ° ♂♂	7.85 ± 0.66	8.05 ± 0.46	5.75 ± 0.50
	26 ° Total	5.55 ± 0.72	5.05 ± 0.54	4.80 ± 0.44
	26 ° ♀♀	2.95 ± 0.42	2.80 ± 0.39	2.20 ± 0.34
	26 ° ♂♂	2.60 ± 0.44	2.25 ± 0.35	2.60 ± 0.37
112/100	18 ° Total	16.25 ± 0.56	14.15 ± 0.80	13.10 ± 0.81
	18 ° ♀♀	8.35 ± 0.53	7.05 ± 0.64	5.90 ± 0.48
	18 ° ♂♂	7.90 ± 0.38	7.10 ± 0.63	7.20 ± 0.54
	26 ° Total	6.79 ± 0.68	6.40 ± 0.69	4.60 ± 0.54
	26 ° ♀♀	3.46 ± 0.49	3.65 ± 0.38	2.60 ± 0.34
	26 ° ♂♂	3.15 ± 0.49	2.75 ± 0.46	2.00 ± 0.32
100/100	18 ° Total	14.65 ± 0.75	12.55 ± 0.62	13.30 ± 0.80
	18 ° ♀♀	7.35 ± 0.54	6.00 ± 0.46	7.20 ± 0.73
	18 ° ♂♂	7.30 ± 0.53	6.55 ± 0.49	6.10 ± 0.49
	26 ° Total	7.10 ± 0.57	5.55 ± 0.62	5.95 ± 0.60
	26 ° ♀♀	2.85 ± 0.36	2.45 ± 0.39	3.15 ± 0.36
	26 ° ♂♂	4.25 ± 0.48	3.10 ± 0.50	2.80 ± 0.42

* Since the *Pgm-1* locus is sex-linked, the male 'heterozygous' class consists of a mixture of *Pgm-1*¹⁰⁴ and *Pgm-1*¹⁰⁰ individuals.

Table 2

Genotypic means and results of the analysis of variance for egg-to-adult survival under optimal conditions. The number of replicates is 20. Bars under means indicate values not significantly different.

Group	PGM genotype			MDH genotype			F from analysis of variance		
	104/104	104/100	100/100	112/112	112/100	100/100	PGM	MDH	PGM × MDH
18 ° Total	15.72	14.03	12.80	14.55	14.50	13.50	10.013***	1.639(ns)	1.815(ns)
18 ° ♀♀	8.03	6.80	6.45	7.33	7.10	6.85	5.451***	0.460(ns)	1.485(ns)
18 ° ♂♂	7.68	7.23	6.35	7.22	7.40	6.65	4.768***	1.612(ns)	1.441(ns)
26 ° Total	6.45	5.67	5.12	5.13	5.90	6.20	3.660*	2.467(ns)	1.000(ns)
26 ° ♀♀	3.12	2.97	2.65	2.65	3.27	2.82	1.120(ns)	2.009(ns)	1.469(ns)
26 ° ♂♂	3.33	2.70	2.47	2.48	2.63	3.38	3.265*	2.797(ns)	0.970(ns)

* Significant at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; (ns) = not significant.

Table 3

Egg-to-adult survival under competitive conditions: mean and standard error of the number of adults emerging from 200 eggs having the given genotype. The mean numbers of emerging females and males are also given. The number of replicates is 8.

MDH genotype	Group	104/104	PGM genotype 104/100*	100/100
112/112	18 ° Total	69.87 ± 7.89	72.50 ± 3.82	67.75 ± 2.75
	18 ° ♀♀	35.62 ± 4.62	36.00 ± 2.17	34.38 ± 1.61
	18 ° ♂♂	35.25 ± 3.57	36.50 ± 2.90	33.37 ± 1.28
	26 ° Total	42.63 ± 7.13	45.38 ± 4.76	49.00 ± 5.63
	26 ° ♀♀	23.38 ± 3.40	23.75 ± 2.76	27.88 ± 3.55
	26 ° ♂♂	19.25 ± 3.42	21.63 ± 2.73	21.13 ± 2.47
112/100	18 ° Total	89.50 ± 2.75	87.50 ± 3.72	73.63 ± 1.84
	18 ° ♀♀	49.63 ± 2.39	45.38 ± 2.84	39.00 ± 1.24
	18 ° ♂♂	39.88 ± 2.31	42.13 ± 2.21	34.63 ± 1.61
	26 ° Total	65.38 ± 3.73	35.75 ± 5.72	24.63 ± 2.67
	26 ° ♀♀	34.88 ± 2.57	18.75 ± 3.36	12.25 ± 1.24
	26 ° ♂♂	30.50 ± 1.60	17.00 ± 2.53	12.38 ± 1.46
100/100	18 ° Total	77.38 ± 4.00	82.25 ± 5.67	68.13 ± 4.48
	18 ° ♀♀	40.00 ± 2.56	39.88 ± 4.15	35.75 ± 2.18
	18 ° ♂♂	37.38 ± 3.06	42.38 ± 2.88	32.38 ± 3.12
	26 ° Total	63.75 ± 3.23	57.88 ± 5.22	59.13 ± 4.16
	26 ° ♀♀	32.63 ± 1.82	27.50 ± 3.12	31.75 ± 2.98
	26 ° ♂♂	31.13 ± 1.82	30.38 ± 3.28	27.38 ± 2.49

* The hemizygous males of this class are a mixture of the *Pgm-I*¹⁰⁴ and *Pgm-I*¹⁰⁰ genotypes.

*Pgm-1*¹⁰⁴ homozygous flies also appears at 26° for total progeny; moreover, at this temperature the heterozygotes survive significantly better than the *Pgm-1*¹⁰⁰ homozygous flies. However, unlike the 18° results, the differences among the flies of different genotypes at 26° are primarily, though not exclusively, due to differential survival among males. The 104 hemizygous males survive significantly better than the 100 males, while the 'heterozygous' class, containing males of both 100 and 104 genotypes, is intermediate in survival and does not significantly differ from either of the other two classes. No significant differences among flies of different *Mdh-2* genotypes occur with respect to egg-to-adult survival at optimal density conditions. Significant interactions between the two loci are also lacking.

The results of the egg-to-adult survival experiments under competitive density conditions are summarized in Table 3. The difference in survival rate between the two temperatures is still apparent, but it is considerably less than it was under optimal conditions. At the competitive density, the overall rate of survival at 18°C is 38.3%, which is about half the rate of survival under optimal conditions (70.9%), while at 26°C the overall survival rate is 24.6%, only slightly less than under optimal conditions (28.7%). One can only speculate about the reasons for this interaction between temperature and density. Perhaps, at 26°C

such a large proportion of the larvae fail to develop due to the high temperature that the competition for food resources at high density is not very great.

As was the case for optimal conditions, a number of differences appear among the genotypes with respect to egg-to-adult survival at competitive density, but the results at the two densities differ in interesting ways. As shown in Table 4, flies of different *Pgm-1* genotypes are significantly heterogeneous with respect to this fitness component, as they were under optimal density conditions. But now at 18°C, the heterozygous flies as well as the 104/104 homozygotes are significantly superior to the 100/100 homozygotes and the heterozygotes survive better, although not significantly so, than the 104/104 homozygotes rather than significantly worse as it was under optimal conditions. The overall differences among the genotypes are largely due to differences among the males.

With respect to the *Mdh-2* genotype there are significant differences at 18°C competitive conditions while there were none under optimal conditions. The heterozygous flies survive significantly better than either one of the homozygotes. This difference is largely due to differential survival of the females. As was the case for the optimal density experiments, there are no significant interactions between loci at 18°C.

Table 4

Genotypic means and results of the analysis of variance for egg-to-adult survival under competitive conditions. The number of replicates is 8. Bars under means indicate values not significantly different.

Group	PGM genotype			MDH genotype			F from analysis of variance		
	104/104	104/100	100/100	112/112	112/100	100/100	PGM	MDH	PGM × MDH
18 ° Total	78.92	80.75	69.83	70.04	83.54	75.92	5.299**	7.105**	0.759(ns)
18 ° ♀♀	41.75	40.42	36.38	35.33	44.67	38.54	2.918(ns)	8.375***	0.724(ns)
18 ° ♂♂	37.17	40.33	33.46	34.71	37.88	37.38	5.077**	1.812(ns)	0.484(ns)
26 ° Total*	57.25	46.33	44.38	45.67	41.92	60.25	6.282**	11.914***	6.559***
26 ° ♀♀*	30.29	23.33	23.96	25.00	21.96	30.63	5.432*	7.086**	6.413***
26 ° ♂♂*	26.96	23.00	20.29	20.67	19.95	29.63	5.361**	13.845***	4.806**

* Significant at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; (ns) = not significant.

* Because of the significant interaction between the two loci, differences between means are not tested at 26 °C.

At 26°C the situation is different. As was found under optimal conditions, there is significant heterogeneity among the *Pgm-1* genotypes. However, unlike the optimal density situation, the *Mdh-2* genotypes are significantly heterogeneous under competitive conditions. Moreover, the combination of marginal temperature and high density induces a significant interaction between the loci which is present when males, females, or total progeny are analyzed. Due to this significant interaction no Student-Newman-Keuls-test was performed to compare means. A cursory observation of the genotype means (Table 4) indicates that flies of the *Pgm-1*^{104/104} genotype have a higher survival rate than those of the alterna-

tive genotypes and that flies of the *Mdh-2*^{100/100} genotype have a much higher survival rate than those with alternative genotypes. This is the only case where the flies with the *Mdh-2*¹⁰⁰ homozygous genotype, the most common one in the natural population, have a substantially higher rate of survival than the *112/112* homozygotes.

The fecundity means are presented in Table 5. This table shows that a high variance is encountered in estimating fecundity (the standard errors are about 10% of the means, while they were only about 5% with respect to survival). Given this high within-genotype variance for both temperatures it is not surprising that the 18° data show all genotypes as statis-

Table 5

Fecundity and male mating capacity. Fecundity entries represent the mean and standard error of the number of eggs laid by a group of three females in eight days. Male mating capacity entries represent the mean and standard error of the number of females inseminated by a male in a 24 hour period. The number of replicates is 12 for fecundity, 16 for male mating capacity at 18 °C and 10 for male mating capacity at 26 °C.

MDH genotype	Group	Fecundity (PGM genotype)			Mating capacity (PGM genotype)	
		104/104	104/100	100/100	104	100
112/112	18 °	578.3 ± 53.0	714.1 ± 70.9	600.3 ± 60.6	8.06 ± 0.39	7.31 ± 0.60
	26 °	699.0 ± 62.3	695.5 ± 36.5	655.3 ± 56.8	7.20 ± 0.36	7.20 ± 0.29
112/100	18 °	627.8 ± 59.7	610.7 ± 54.6	594.4 ± 60.2	7.56 ± 0.97	7.31 ± 0.79
	26 °	527.4 ± 74.3	507.3 ± 50.8	510.6 ± 59.5	7.50 ± 0.65	5.80 ± 0.92
100/100	18 °	656.3 ± 46.2	675.7 ± 58.3	651.5 ± 42.8	8.63 ± 0.58	8.63 ± 0.47
	26 °	579.5 ± 68.6	504.7 ± 52.5	665.5 ± 61.8	5.80 ± 1.06	6.50 ± 0.88

Table 6

Genotypic means and results of the analysis of variance for fecundity and male mating capacity. The number of replicates for fecundity is 12; for mating capacity it is 16 at 18 °C and 10 at 26 °C. Bars under means indicate values not significantly different.

Fitness component	PGM genotype			MDH genotype			F from analysis of variance		
	104/104	104/100	100/100	112/112	112/100	100/100	PGM	MDH	PGM × MDH
Fecundity 18 °	620.8	666.8	615.4	630.9	611.0	661.2	0.742(ns)	0.594(ns)	0.521(ns)
Fecundity 26 °	602.0	659.2	610.5	683.3	515.0	583.3	0.409(ns)	6.159***	0.825(ns)
Mating capacity 18 °	8.08		7.75	7.68	7.43	8.63	0.378(ns)	1.777(ns)	0.165(ns)
Mating capacity 26 °	6.83		6.50	7.20	6.65	6.15	0.284(ns)	0.939(ns)	1.295(ns)

*** Significant at $P < 0.001$; (ns) = not significant.

tically equivalent (Table 6). Females of different *Mdh-2* genotypes are, however, heterogeneous with respect to fecundity at 26°. Females of the *Mdh-2*^{112/112} genotype are appreciably more fecund at this temperature than are females with alternative genotypes. Females of the different *Pgm-1* genotypes do not vary significantly in fecundity at 26° and there is no significant interaction between the loci at either temperature.

The mean number of females inseminated per male are shown in Table 5. No significant heterogeneity is found among males of alternative genotypes for either locus at either temperature (Table 6).

Discussion

The *Pgm-1* and *Mdh-2* loci are excellent subjects for an examination of components of fitness. The *Pgm-1* and *Mdh-2* loci of *Drosophila pseudoobscura* exhibit markedly different patterns of variation in natural populations. *Pgm-1* is a highly polymorphic locus in almost all populations of *D. pseudoobscura*; the *Mdh-2* locus is much less polymorphic in all populations. Both loci in *D. pseudoobscura* have two alleles which account for most of the variation in the population, and neither locus is tied up in inversions. (No fourth-chromosome inversions exist in natural populations; in the X-chromosome the only common inversion is the 'sex-ratio' inversion which results in exclusively female progeny.)

Previous studies of components of fitness at allozyme loci in *Drosophila* point out several factors which must be taken into account. Jones & Yamazaki (1974) demonstrated the importance of using large numbers of independently derived chromosomes. The use of 20 independent lines of each of the four original doubly-homozygous strains in the present experiments assures that more than 95% of the variation in the natural population is represented in the experimental population (Nei et al., 1975). Another factor which may be important in experimental results is the 'age' of the experimental strains. Stocks recently derived from natural populations (Nei et al., 1975) may give far different results in selection or fitness studies than stocks that have been maintained in the laboratory for prolonged periods (Fontdevila, 1973). The stocks used in these experiments were six generations removed from the natural population. A third factor to consider is the availability of stocks. A complete

design requires stocks such that all possible genotypic combinations are available for evaluation. Use of a complete design greatly simplifies data analysis and allows for a test of interaction among the loci under study.

In general the results of this study confirm the previously reported findings of Marinković & Ayala (1975a, b). Flies of no one genotype at either locus are superior for all components of fitness; and the relative order of genotypes changes with respect to a particular component of fitness as conditions vary. The number of components of fitness showing heterogeneity among flies of different genotypes and the number of reversals between changes in conditions are fewer in these experiments than in those of Marinković & Ayala (1975a, b). These differences in results may be due to slight variation in experimental design, or to the use of flies from a different natural population, or to the larger number of independently derived chromosomes employed in our study.

The *Mdh-2* and *Pgm-1* loci, which display different degrees of polymorphism in natural populations, also exhibit differences in the response of flies with alternative genotypes to changing environmental conditions. Flies of the different *Mdh-2* genotypes are equivalent for five of the eight combinations of environmental conditions and fitness components tested. Conditions which elicit heterogeneity among flies of the alternative *Mdh-2* genotypes involve high densities for egg-to-adult survival at either 18° or 26°C and the marginal temperature of 26°C for fecundity. Increased density not only causes heterogeneity for egg-to-adult survival but also affects the sexes differently. At 18°C, females heterozygous for the *Mdh-2* locus have a higher rate of survival than homozygous females while this is not so under optimal density conditions.

Flies of the alternative *Pgm-1* genotypes, on the other hand, show heterogeneity for egg-to-adult survival under all temperature and density conditions tested. As noted for the *Mdh-2* locus, changes in conditions affect the sexes differently. At 18° optimum density conditions, flies of both sexes are significantly different in egg-to-adult survival; however, at 18° competitive and 26° optimum conditions, only males of the alternative genotypes show significant differences in survival.

The differential response of flies of different sex with respect to survival demonstrates that genotypes

at a particular locus may be influenced by their genetic background (males are hemizygous at all sex-linked loci). The heterogeneity in egg-to-adult survival of males carrying alternative *Pgm-1* alleles might be explained in several ways. Actual differences between the *Pgm-1*¹⁰⁰ and *Pgm-1*¹⁰⁴ alleles are one possibility. However, the lack of significant heterogeneity among females under the same conditions suggests that this might not be the case. Another possibility is a non-equilibrium association of alternative *Pgm-1* alleles with sex-linked deleterious or lethal genes which affect egg hatchability. Other studies which demonstrated equivalent survival of male larvae from first instar to adult at an intermediate density (Snyder & Ayala, 1979) suggest this is a strong possibility. Either differential enzyme action in males and females or heterogeneity of *Pgm-1* genotypes due to linked loci would indicate the influence of other loci on the *Pgm-1* locus. Influences of these two types are quite distinct. It is impossible to differentiate among the several possibilities with the available data, although the differences in survival of males and females of alternative *Mdh-2* genotypes at 18° competitive conditions does suggest an influence of the genetic background on genotypic function.

Evaluating the effects of linkage on the results of these experiments is difficult. Clearly selection at loci closely linked to the *Mdh-2* and *Pgm-1* markers would be indistinguishable from selection at the markers themselves. The design of these experiments, which involved the use of large numbers of independently derived chromosomes, ensures that more than 95% of all the genetic variation present in the natural population is represented in the experimental strains. The use of chromosomally heterozygous flies for all measurements should eliminate disequilibrium effects created by establishing single-female lines. Any linkage disequilibrium present in the experimental population should reflect that of the natural population. Thus, if the selection effects observed at the *Pgm-1* and *Mdh-2* loci are due not to the loci per se but to loci with which they are associated in linkage disequilibrium, similar selection effects would also affect these loci in the natural population.

The only evidence in this experiment suggesting major linkage effects is the interaction of the *Mdh-2* and *Pgm-1* loci for egg-to-adult survival at 26° competitive conditions. Given the fact that the enzymes encoded by these two loci function in different meta-

bolic pathways and use different electron acceptors, it seems plausible that the interaction at 26° competitive density conditions is due to loci linked to one or both of the markers.

Drosophila pseudoobscura possessing different *Pgm-1* and *Mdh-2* genotypes are clearly not equivalent for all components of fitness. Neither a consistent heterozygote advantage for all components of fitness nor an overall advantage of the heterozygous genotypes (marginal overdominance; Wallace, 1968) occur. Nevertheless, there occur shifts in relative fitness which correspond to environmental changes in temperature and density. The complete array of relative fitnesses under all conditions can, of course, never be observed in the laboratory. It is unlikely that one could obtain in the laboratory conditions approximating those accounting for the maintenance of balanced polymorphisms in natural populations. However, the demonstration of fitness differences among genotypes and of fitness shifts associated with changing environmental conditions do suggest that selection plays an important role in maintaining variability at the *Mdh-2* and *Pgm-1* loci. Whether selection acts directly on the loci themselves or through the blocks of genes with which they are associated cannot now be determined.

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References

- Ayala, F.J., J.R. Powell, M.L. Tracey, C.A. Mourão & S. Pérez-Salas (1972). Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics* 70: 113-139.
- Fontdevila, A. (1973). Experimental studies of allozyme fitness in population cages of *Drosophila pseudoobscura*. Proc. XIII Int. Conf. Genet. *Genetics* 74: S82 (Abst.).
- Jones, J.S. & T. Yamazaki (1974). Genetic background and the fitness of allozymes. *Genetics* 78: 1185-1189.
- Kojima, K. & Y.N. Tobari (1969). The pattern of viability changes associated with genotype frequency at the alcohol dehydrogenase locus in a population of *Drosophila melanogaster*. *Genetics* 61: 201-209.
- Kojima, K. & K.M. Yarbrough (1967). Frequency-dependent selection at the esterase-6 locus in *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* 57: 645-649.
- Marinković, D. (1967). Genetic loads affecting fertility in natural populations of *Drosophila pseudoobscura*. *Genetics* 57: 701-709.

- Marinković, D. & F.J. Ayala (1975a). Fitness of allozyme variants in *Drosophila pseudoobscura*. I. Selection at the Pgm-1 and Me-2 loci. *Genetics* 79: 85-95.
- Marinković, D. & F.J. Ayala (1975b). Fitness of allozyme variants in *Drosophila pseudoobscura*. II. Selection at the Est-5, Odh and Mdh-2 loci. *Genet. Res.* 24: 137-149.
- Marinković, D. & F.J. Ayala (1977). Fitness of allozyme variants in *Drosophila pseudoobscura*. III. Possible factors contributing to the maintenance of polymorphisms in nature. *Genetics* 47: 65-70.
- Prout, T. (1965). The estimation of fitnesses from genotypic frequencies. *Evolution* 19: 546-551.
- Snyder, T.P. & F.J. Ayala (1979). Frequency-dependent selection at the Pgm-1 locus of *Drosophila pseudoobscura*. *Genetics* 92: in press.
- Sokal, R.R. & F.J. Rohlf (1969). *Biometry*. W.H. Freeman and Co., San Francisco. 776 pp.
- Yamazaki, T. (1971). Measurement of fitness of the esterase-5 locus in *Drosophila pseudoobscura*. *Genetics* 67: 579-603.