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Fitness of allozyme variants in *Drosophila pseudoobscura*

II. Selection at the *Est-5*, *Odh* and *Mdh-2* loci*

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SUMMARY

We have studied the effects on fitness of allelic variation at three gene loci (*Est-5*, *Odh*, and *Mdh-2*) coding for enzymes in *Drosophila pseudoobscura*. Genotype has a significant effect on fitness for all six parameters measured (female fecundity, male mating capacity, egg-to-adult survival under near-optimal and under competitive conditions, and rate of development under near-optimal and under competitive conditions). No single genotype is best for all six fitness parameters; rather, genotypes with superior performance during a certain stage of the life-cycle may have low fitness at some other stage, or in different environmental conditions. Heterozygotes are sometimes best when all fitness parameters are considered. There are significant interactions between loci. The various forms of balancing selection uncovered in our experiments may account for the polymorphisms occurring in natural populations of *D. pseudoobscura* at the three loci studied.

1. INTRODUCTION

We have previously reported our studies in *Drosophila pseudoobscura* of the effects of allozyme variants coded by alleles at two loci (*Pgm-1* in the X chromosome and *Me-2* in the second chromosome) on a variety of fitness components (Marinković & Ayala, in the press). Of the six genotypic combinations tested, none is the best for all fitness components. Rather, the relative fitnesses of genotypes are often reversed when different parameters are considered, or when they are studied in different conditions. The *Pgm-1* and *Me-2* loci are very polymorphic in natural populations of *D. pseudoobscura*; moreover, the allelic frequencies oscillate cyclically throughout the seasons (Dobzhansky & Ayala 1973). The kinds of balancing selection found in the laboratory by Marinković and Ayala may account for the persistence and fluctuations of the natural polymorphisms.

In the present experiments we have extended our study to three additional loci coding for enzymes in *D. pseudoobscura*. One locus, esterase-5 (*Est-5*, X chromosome) is highly polymorphic in natural populations (more than 50% of the individuals are heterozygotes); while the other two, octanol dehydrogenase (*Odh*,

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second chromosome) and malate dehydrogenase-2 (*Mdh-2*, fourth chromosome) are only moderately polymorphic (about 10 % of the individuals are heterozygous in natural populations). The effect of the genotype on six fitness components has been studied in 11 different genotype combinations of alleles at the three loci.

2. MATERIAL AND METHODS

Eighty-three strains of *D. pseudoobscura*, homozygous for particular alleles at the *Est-5*, *Odh*, and *Mdh-2* loci, were obtained in our laboratory during the summer of 1973, in one to three generations by a series of more than 3000 single-pair matings. Each of the 83 strains originated from a different wild-type female, collected in the spring of 1973 (a few strains descended from flies collected in the fall of 1972 were also used) at McDonald Ranch, Napa County, California. The flies were collected in a forest dominated by pine trees, using banana baits 10–20 m apart, the total area covered having a diameter of 200 m. There were seven groups of such strains, as follows:

	A	B	C	D	E	F	G
Est-5	100/100	104/104	100/100	100/100	104/104	104/104	104/104
Odh	100/100	100/100	104/104	100/100	104/104	100/100	104/104
Mdh-2	100/100	100/100	100/100	112/112	100/100	112/112	112/112
No. of strains	19	18	11	12	14	5	4

To randomize the genotypes, a series of crosses between the strains of each group have been performed. First, we have obtained 20 combined strains in each of the groups A, B, C, D, and E, 10 strains in group F, and 6 in group G – each combined strain obtained by crossing a different pair of the original strains. In the following generation, crosses have been made between pairs of such synthetic strains within the same group (e.g. $A_{1,2} \times A_{3,4}$; or $B_{7,8} \times B_{9,10}$; or $G_{2,3} \times G_{4,1}$, etc.), and also between females from group A and males from groups B, C, D, or G. Consequently, four new genotypes have been produced (16 synthetic strains of each), three of them heterozygous either at the *Est-5* (A × B), the *Odh* (A × C), or the *Mdh-2* (A × D) locus, and one genotype heterozygous at all three loci (A × G). The 11 genotypes with a total of 170 synthetic strains used in the experiment are as follows:

	A	B	C	D	E	F	G
Est-5	100/100	104/104	100/100	100/100	104/104	104/104	104/104
Odh	100/100	100/100	104/104	100/100	104/104	100/100	104/104
Mdh-2	100/100	100/100	100/100	112/112	100/100	112/112	112/112
No. of synthetic strains	20	20	20	20	20	5	1

	A × B	A × C	A × D	A × G
Est-5	100/104	100/100	100/100	100/104
Odh	100/100	100/104	100/100	100/104
Mdh-2	100/100	100/100	100/112	100/112
No. of synthetic strains	16	16	16	16

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Egg-to-adult survival and rate of development under near optimal conditions (100 eggs in a half-pint culture), and under competitive conditions (200 eggs in an 11 mm-diameter vial) were measured in each of the 170 strains, all raised simultaneously. Fecundity was studied in females raised under optimal conditions, by counting the number of eggs laid from the 6th to the 13th day after emergence by groups of 3 females and 3 males of a given strain. Mating capacity of males was studied in 3-day-old males raised under optimal conditions as follows. Each of 25 males of a given genotype was placed individually in a culture bottle with 8 virgin females from a common pool of all strains; the number of females inseminated by each male during 24 h was determined by inspecting the reproductive tracts of the females for presence of sperm.

All cultures were kept in a constant temperature incubator at $25 \pm 0{\cdot}5$ °C and *c.* 70 % relative humidity, with light switched on between 7 a.m. and 7 p.m. As food we have used standard agar-cornmeal-molasses *Drosophila* medium.

3. RESULTS

Table 1 gives the means and standard errors of the six fitness parameters studied for each of the eleven genotypes. Each genotype has a specific combination of two alleles at the *Est-5*, *Odh* and *Mdh-2* loci. In seven groups (A, B, C, D, E, F and G), flies are homozygous at all three loci, either for the most common allele in nature (100), or for a less common allele (104 for *Est-5* and *Odh*; 112 for *Mdh-2*). In three groups (A × B, A × C and A × D) flies are heterozygous at one, and homozygous at other two loci. In one group (A × G) flies are heterozygous at all three loci.

Statistical significance of the effect of genotype on each of the six fitness components is tested by one-way analyses of variance. The differences among the eleven genotypes are highly significant in all six fitness parameters: at the 0·025 level for egg-to-adult survival under near optimal conditions, and at the 0·001 level for the other five components of fitness.

Table 2 compares the performance of flies which are homozygous at the three loci with that of flies heterozygous at either one locus or at all three loci. Heterozygotes are ostensibly superior in male mating capacity ($4{\cdot}63 \pm 0{\cdot}17$ versus $3{\cdot}94 \pm 0{\cdot}16$ inseminated females per male, per 24 h; $t_{243} = 2{\cdot}96$, $P < 0{\cdot}01$) and in developmental rate under competitive conditions ($15{\cdot}97 \pm 0{\cdot}19$ versus $16{\cdot}66 \pm 0{\cdot}14$ days; $t_{86} = 2{\cdot}92$, $P < 0{\cdot}01$). However, the homozygotes as a group are superior in developmental rate under optimal conditions ($13{\cdot}54 \pm 0{\cdot}03$ versus $13{\cdot}73 \pm 0{\cdot}04$ days; $t_{157} = 3{\cdot}80$, $P < 0{\cdot}001$). The differences in female fecundity and in egg-to-adult survival are not statistically significant.

Among the homozygotes (see Table 1), the carriers of the allele most common in nature at all three loci (*Est-5*,^{100} *Odh*,^{100} and *Mdh-2*,^{100} group A) have high female fecundity, male mating capacity, and egg-to-adult survival and rate of development under near-optimal conditions. Under competitive conditions, however, their survival and rate of development are not significantly different from the average of all homozygotes. The homozygotes for all three rare alleles (*Est-5*,^{104}

Table 1. *Means and standard errors for six fitness parameters in eleven genetically different strains of* Drosophila pseudoobscura

(The numbers of replications are given in parentheses.)

	Strains												
	A	B	C	D	E	F	G	A×B	A×C	A×D	A×G	Degrees of freedom	*F*
Genotype *Est-5*†	100/100	104/104	100/100	100/100	104/104	104/104	104/104	100/104	100/100	100/100	100/104		
Genotype *Odh*	100/100	100/100	104/104	100/100	104/104	100/100	104/104	100/100	100/104	100/100	100/104		
Genotype *Mdh-2*	100/100	100/100	100/100	112/112	100/100	112/112	112/112	100/100	100/100	100/112	100/112		
Female fecundity‡	324 ±10 (15)	332 ±15 (15)	296 ±14 (15)	302 ±16 (15)	205 ±14 (15)	233 ±20 (15)	304 ±12 (15)	304 ±12 (10)	298 ±26 (10)	264 ±17 (10)	310 ±16 (15)	10, 139	6·52***
% egg-to-adult survival:													
Optimal conditions	87·0 ±2·2 (20)	67·2 ±6·0 (20)	82·0 ±2·1 (20)	76·3 ±4·4 (20)	81·3 ±5·8 (12)	73·9 ±4·6 (8)	74·1 ±3·2 (9)	76·5 ±7·4 (13)	89·4 ±2·6 (11)	81·5 ±3·4 (13)	77·1 ±3·8 (16)	10, 151	2·09*
Competitive conditions	40·8 ±1·6 (10)	39·5 ±3·5 (10)	39·6 ±3·1 (10)	45·7 ±2·0 (10)	51·5 ±3·7 (7)	25·7 ±2·9 (7)	31·0 ±8·1 (4)	39·9 ±2·9 (6)	37·5 ±3·7 (7)	44·1 ±4·4 (5)	37·4 ±2·4 (12)	10, 77	3·95***
Rate of development (in days):													
Optimal conditions	13·28 ±0·06 (20)	13·54 ±0·07 (18)	13·69 ±0·09 (20)	13·73 ±0·06 (20)	13·16 ±0·07 (12)	13·76 ±0·07 (8)	13·63 ±0·10 (9)	13·83 ±0·11 (12)	13·84 ±0·07 (11)	13·51 ±0·08 (13)	13·76 ±0·08 (16)	10,148	7·19***
Competitive conditions	17·35 ±0·27 (10)	16·29 ±0·54 (10)	17·56 ±0·47 (10)	17·05 ±0·35 (10)	15·84 ±0·37 (7)	15·83 ±0·23 (7)	16·78 ±0·15 (4)	17·36 ±0·31 (6)	15·90 ±0·60 (7)	15·48 ±0·27 (5)	15·12 ±0·20 (12)	10, 77	4·95***
Male mating capacity‡	4·75 ±0·54 (25)	2·77 ±0·50 (25)	5·12 ±0·38 (23)	4·34 ±0·40 (25)	3·63 ±0·32 (24)	3·73 ±0·37 (24)	3·21 ±0·37 (23)	— — —	5·40 ±0·29 (25)	4·65 ±0·46 (26)	3·83 ±0·42 (25)	9, 235	4·16***

* Statistically significant $P < 0{\cdot}025$; *** $P < 0{\cdot}001$.

† The genotype of males at the *Est-5* locus is 100/Y in strains A, C, D, A×B, A×C, A×D and A×G.

‡ 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 h.

Odh-2,[104] and *Mdh-2*[112] group G) have low male mating capacity and egg-to-adult survival, both under optimal and competitive conditions, but in the other fitness parameters they are not ostensibly different from the average of all studied homozygotes. Heterozygotes for all three loci have average fitness for most parameters, but their developmental rate under competitive conditions is the best among the eleven genotypic groups.

Table 2. *Means and standard errors for six fitness parameters in homozygotes (strains A, B, C, D, E, F and G) and in heterozygotes (A × B, A × C, A × D and A × G) at the* Est-5, Odh, *and/or* Mdh-2 *loci*

(The number of replications is given in parentheses.)

	Heterozygotes*	Homozygotes
Female fecundity†	294 ± 9 (45)	285 ± 5 (105)
% egg-to-adult survival:		
Optimal conditions	81·1 ± 2·3 (53)	77·4 ± 1·6 (109)
Competitive conditions	39·7 ± 1·7 (30)	39·1 ± 1·5 (58)
Rate of development (in days):		
Optimal conditions	13·73 ± 04 (52)	13·54 ± 0·03 (107)
Competitive conditions	15·97 ± 0·19 (30)	16·66 ± 0·14 (58)
Mating capacity of males	4·63 ± 0·17 (76)	3·94 ± 0·16 (169)

* Since *Est-5* is a sex-linked locus, the heterozygosity relates only to autosomal *Odh* and *Mdh-2* loci.

† See footnote ‡ in Table 1.

Table 3 gives the means and standard errors of the six fitness parameters for the *Est-5* genotypes. The experimental strains represent random combinations of 42 wild chromosomes carrying allele 100, and 41 wild chromosomes carrying allele 105. The *F*-test for overall differences among the three genotypes (*Est-5*,[100/100] *Est-5*[100/100], and *Est-5*[100/104]) is statistically significant for five out of the six parameters. The only exception is egg-to-adult survival under competitive conditions. In three parameters (egg-to-adult survival under both conditions and developmental rate under competitive conditions), the heterozygotes have intermediate fitness values. However, they have the best female fecundity and developmental rate under near-optimal conditions. The homozygotes for the allele most common in nature (*Est-5*[100/100]) are superior to the homozygotes for the rarer allele (*Est-5*[104/104], in fecundity ($t_{123} = 2{\cdot}65$, $P < 0{\cdot}01$), egg-to-adult survival under optimal conditions ($t_{131} = 3{\cdot}18$, $P < 0{\cdot}01$) and male mating capacity ($t_{243} = 5{\cdot}11$, $P < 0{\cdot}001$); they are somewhat inferior in rate of development, both under near-optimal and under competitive conditions, but these latter differences are not statistically significant.

Table 4 gives the means and standard errors for the three *Odh* genotypes. Fifty-four wild chromosomes carrying allele 100, and 29 carrying allele 104, were used to establish the experimental strains. *F*-test values are statistically significant for fecundity ($P < 0{\cdot}05$) and for developmental rate under near-optimal and under competitive conditions ($P < 0{\cdot}001$). The heterozygotes are the best in fecundity,

Table 3. *Means and standard errors for six fitness parameters in three genotypes at the sex-linked* Est-5 *locus of* Drosophila pseudoobscura

(The number of replications is given in parentheses.)

	Genotypes			Degrees of freedom	F
	100/100	100/104	104/104		
Female fecundity‡	297 ± 7 (65)	307 ± 10 (25)	268 ± 8 (60)	2, 147	4·67**
% egg-to-adult survival:					
Optimal conditions	83·2 ± 1·4 (94)	76·8 ± 4·2 (29)	74·1 ± 2·5 (49)	2, 159	4·98**
Competitive conditions	41·5 ± 1·4 (42)	38·7 ± 1·9 (18)	36·9 ± 2·5 (28)	2, 85	1·29
Rate of development (in days):					
Optimal conditions	13·61 ± 0·03 (84)	13·80 ± 0·07 (28)	13·52 ± 0·04 (47)	2, 156	9·71**
Competitive conditions	16·67 ± 0·18 (42)	16·24 ± 0·18 (18)	16·19 ± 0·18 (28)	2, 85	5·16**
Mating capacity of males‡	4·68 ± 0·17 (149)	†	3·34 ± 0·20 (96)	1, 243	24·65***

** Statistically significant, $P < 0·01$; *** $P < 0·001$.
† No males heterozygous for both alleles exist since *Est-5* is a sex-linked locus.
‡ See footnote ‡ in Table 1.

Table 4. *Means and standard errors for six fitness parameters in three genotypes at the second chromosome* Odh *locus of* Drosophila pseudoobscura

(The number of replications is given in parentheses.)

	Genotypes			Degrees of freedom	F
	100/100	100/104	104/104		
Female fecundity†	293 ± 6 (80)	304 ± 15 (25)	268 ± 7 (45)	2, 147	3·11*
% egg-to-adult survival:					
Optimal conditions	77·1 ± 2·0 (94)	83·3 ± 2·3 (27)	79·1 ± 2·3 (41)	2, 159	1·06
Competitive conditions	39·3 ± 1·2 (48)	37·5 ± 2·2 (19)	40·7 ± 3·1 (21)	2, 85	1·09
Rate of development (in days):					
Optimal conditions	13·61 ± 0·03 (91)	13·80 ± 0·05 (27)	13·49 ± 0·05 (41)	2, 156	14·18***
Competitive conditions	16·56 ± 0·14 (48)	15·51 ± 0·32 (19)	16·73 ± 0·21 (21)	2, 85	8·44***
Mating capacity of males†	4·05 ± 0·20 (125)	4·62 ± 0·25 (50)	3·99 ± 0·21 (70)	2, 242	1·51

* Statistically significant, $P < 0·05$; *** $P < 0·001$.
† See footnote ‡ in Table 1.

survival under near-optimal conditions, male mating capacity, and significantly so in developmental rate under competitive conditions; they have, however, the lowest survival under competitive conditions, and significantly slower developmental rate under near-optimal conditions than the other two genotypes. The homozygotes for the allele most common in nature ($Odh^{100/100}$) are superior to the homozygotes for the rarer allele ($Odh^{104/104}$) in fecundity ($t_{123} = 2·51$, $P < 0·02$), but are inferior to them in rate of development under near-optimal conditions ($t_{130} = 2·06$, $P < 0·05$).

Table 5 gives the means and standard errors for the *Mdh-2* genotypes. The experimental strains have random combinations of 62 wild chromosomes carrying allele 100, and 21 wild chromosomes carrying allele 112. *F*-test values are statistically significant only for rate of development, both under near-optimal ($P < 0{\cdot}01$) and under competitive conditions ($P < 0{\cdot}001$). Heterozygotes have intermediate fitness values in four of the fitness parameters, but they are significantly superior to the other two genotypes in rate of development under competitive conditions.

Table 5. *Means and standard errors for six fitness parameters in three genotypes at the fourth chromosome* Mdh-2 *locus of* Drosophila pseudoobscura

(The number of replications is given in parentheses.)

	Genotypes			Degrees of freedom	*F*
	100/100	100/112	112/112		
Female fecundity†	282 ± 7 (80)	287 ± 12 (25)	280 ± 9 (45)	147, 2	1·91
% egg-to-adult survival:					
Optimal conditions	80·6 ± 2·0 (96)	79·3 ± 2·5 (29)	74·8 ± 2·4 (37)	2, 159	1·09
Competitive conditions	41·5 ± 1·3 (50)	40·8 ± 2·5 (17)	34·1 ± 2·9 (21)	2, 85	1·72
Rate of development (in days):					
Optimal conditions	13·56 ± 0·03 (93)	13·64 ± 0·06 (29)	13·71 ± 0·04 (37)	2, 156	5·20**
Competitive conditions	16·72 ± 0·18 (50)	15·30 ± 0·17 (17)	16·55 ± 0·15 (21)	2, 85	10·44***
Mating capacity of males†	4·33 ± 0·19 (122)	4·24 ± 0·31 (51)	3·76 ± 0·22 (72)	2, 242	1·51

** Statistically significant, $P < 0{\cdot}10$; *** $P < 0{\cdot}001$.
† See footnote ‡ in Table 1.

The homozygotes ($Mdh\text{-}2^{100/100}$ and $Mdh\text{-}2^{112/112}$) are significantly different only in developmental rate under near-optimal conditions: the carriers of the allele most common in nature are again superior to the carriers of the rarer allele ($t_{128} = 3{\cdot}00$, $P < 0{\cdot}01$).

To test for interaction between genotypes at the three loci, we have made two-way analyses of variance. For each of three kinds of interaction (*Est* × *Odh*, *Est* × *Mdh* and *Mdh* × *Odh*) four groups of genotypes have been used: homozygotes at both loci for the common alleles (100), one locus homozygous for the common and another for the rare allele and vice versa, and both loci homozygous for the rare alleles (104 or 112). The results are presented in Table 6. Between the *Est-5* and *Odh* loci the interaction is statistically significant only for the rate of development under near-optimal conditions ($F_{1,118} = 9{\cdot}92$; $P < 0{\cdot}01$). The overall variation is significant for all six fitness components, but in four out of six components this is due to variation at the *Est-5* locus.

Between the *Est-5* and *Mdh-2* loci the interaction is statistically significant for egg-to-adult survival under competitive conditions ($F_{1,63} = 19{\cdot}55$; $P < 0{\cdot}001$). The overall variation is statistically significant for five of the six fitness parameters. The effect of variation at the *Est-5* locus is significant for four parameters, and at the *Mdh-2* locus for the other two (developmental rate under near-optimal conditions, and egg-to-adult survival under competitive conditions).

Table 6. *Two-way analyses of variance for six fitness parameters between loci* Est-5 × Odh (*strains A, D, A × D; B, F; C; E and G*), Est-5 × Mdh-2 (*A, C, A × C; B, E; D; F and G*) *and* Odh × Mdh-2 (*A, B, A × B; D, F; C, E; G*)

(The degrees of freedom are given in parentheses.)

	Est-5 × Odh				*Est-5 × Mdh-2*				*Mdh-2 × Odh*			
	F_x	F_{Est}	F_{Odh}	F_{ExO}	F_x	F_{Est}	F_{Mdh}	F_{ExM}	F_x	F_{Mdh}	F_{Odh}	F_{MxO}
Female fecundity	2·93*	6·16*	3·65	1·02	2·79*	8·30**	0·67	0·61	8·90***	0·98	6·51*	19·22***
	(3, 111)	(1, 113)	(1, 113)	(1, 113)	(3, 111)	(1, 113)	(1, 113)	(1, 113)	(3, 111)	(1, 113)	(1, 113)	(1, 113)
% egg-to-adult survival												
Optimal conditions	3·69*	7·64**	0·72	2·73	4·75**	9·91***	2·42	1·89	1·41	0·84	0·95	0·34
	(3, 118)	(1, 122)	(1, 122)	(1, 122)	(3, 116)	(1, 118)	(1, 118)	(1, 118)	(118, 3)	(1, 120)	(1, 120)	(1, 120)
Competitive conditions	3·31*	0·05	0·01	0·11	8·55***	1·97	4·13*	19·55***	2·27	3·87	1·01	1·94
	(3, 59)	(1, 61)	(1, 61)	(1, 61)	(3, 61)	(1, 63)	(1, 63)	(1, 63)	(3, 60)	(1, 62)	(1, 62)	(1, 62)
Rate of development:												
Optimal conditions	4·71**	0·84	3·36	9·92**	4·92**	3·04	10·09**	1·64	3·21*	9·19**	1·71	1·26
	(3, 116)	(1, 118)	(1, 118)	(1, 118)	(3, 114)	(1, 116)	(1, 116)	(1, 116)	(3, 115)	(1, 117)	(1, 117)	(1, 117)
Competitive conditions	3·68*	8·71**	0·75	1·60	2·66	7·88**	0·06	0·12	2·98	0·85	0·02	0·14
	(3, 59)	(1, 61)	(1, 61)	(1, 61)	(3, 61)	(1, 63)	(1, 63)	(1, 63)	(3, 60)	(1, 62)	(1, 62)	(1, 62)
Mating capacity of males	7·39***	20·80***	0·06	1·24	10·86***	29·56***	3·35	0·32	1·59	0·65	0·05	4·07*
	(3, 191)	(1, 193)	(1, 193)	(1, 193)	(3, 190)	(1, 192)	(1, 192)	(1, 192)	(3, 165)	(1, 167)	(1, 167)	(1, 167)

* Statistically significant $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Between the *Mdh-2* and *Odh* loci there are two significant interactions: female fecundity ($F_{1,113} = 19{\cdot}22$; $P < 0{\cdot}001$) and male mating capacity ($F_{1,167} = 4{\cdot}07$; $P < 0{\cdot}05$). The overall variation is significant only for fecundity and for developmental rate under near-optimal conditions. For fecundity it is due to the effect of variation at the *Odh* locus and to interaction between the two loci; for developmental rate it is due to the *Mdh-2* locus.

4. DISCUSSION

In a previous experiment (Marinković & Ayala, in the press), we have examined electrophoretic variants at the *Pgm-1* and *Me-2* loci of *D. pseudoobscura*, and found that genotype has a statistically significant effect in four of the seven fitness components studied. The overall variation among the six genotypes studied was statistically significant for female fecundity, hatchability of eggs, egg-to-adult survival under optimal conditions, and male mating capacity. In the present experiment we have extended our study to another group of eleven genotypes consisting of combinations of two allelic variants at each of three loci (*Est-5*, *Odh* and *Mdh-2*). Relative to the previous study, we have increased the number of strains studied as well as the number of replications, and have found that the genotype has a statistically significant effect in every one of the six fitness parameters studied. The fitness parameters are female fecundity, egg-to-adult survival under optimal and under competitive conditions, rate of development under optimal and under competitive conditions, and male mating capacity.

Our experiments show that the adaptive values of genotypes may depend on many factors. We shall point out some of them. As we have suggested earlier (Marinković & Ayala, in the press), some of these factors, alone or in combination, may contribute to the maintenance of allozyme polymorphisms in natural populations.

1. *Counteracting selective advantages of the genotypes at different stages of the life cycle.* Genotypes which are the best for a particular fitness component, often have low values for some other fitness parameters. For instance (Table 1), flies homozygous for *Est-5*$^{104/104}$ *Odh*$^{100/100}$ and *Mdh-2*$^{100/100}$ (group B) have the highest female fecundity among the eleven genotypes; but they are the worst for egg-to-adult survival under near-optimal conditions, and for male mating. Flies from group A × C (*Est-5*$^{100/100}$ *Odh*$^{100/104}$ *Mdh-2*$^{100/100}$) have the best egg-to-adult survival and male mating capacity under near optimal conditions, but their developmental rate under the same conditions is the slowest one. Flies from group E (*Est-5*$^{104/104}$ *Odh*$^{100/104}$ *Mdh-2*$^{100/100}$) have very high egg-to-adult survival and one of the fastest developmental rates; their female fecundity, however, is the lowest one – about 40 % lower than in groups A and B.

2. *Marginal heterosis* (*Wallace, 1968*)*: superiority of the heterozygotes when all fitness components are considered.* Often one of the homozygotes is as good as, or better than the heterozygotes for a given fitness component; but alternative homozygotes are superior for different fitness components resulting in net superiority of the heterozygotes. This phenomenon was observed at the *Pgm-1* and *Me-2*

loci (Marinković & Ayala, in the press), and may also occur in the present experiment, at least for some loci. From Tables 3–5 we can calculate the number of adults emerging per egg laying female. This is simply done by multiplying the number of eggs laid per female for 7 days by the survival rate from egg-to-adult. The results are shown in Table 7. The heterozygous genotype is clearly superior for the *Odh* locus under optimal conditions, and is about as good as the best homozygotes in several other cases. Moreover at the *Odh* locus the heteroyzgous males have greater mating capacity than males homozygous for either allele.

Table 7. *Number of adults emerging and mating capcity of males for nine 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	
Est-5$^{100/100}$	247	123	4·7
Est-5$^{100/104}$	236	119	*
Est-5$^{104/104}$	199	99	3·3
Odh$^{100/100}$	226	115	4·1
Odh$^{100/104}$	253	114	4·6
Odh$^{104/104}$	212	109	4·0
Mdh-2$^{100/100}$	227	117	4·3
Mdh-2$^{100/112}$	228	117	4·2
Mdh-2$^{112/112}$	209	95	3·8

* No males heterozygous for both alleles exist at the *Est-5* locus, since the locus is sex-linked.

3. *Fitness interactions between different loci.* Statistically significant interactions between genotypes at different loci exist for some fitness components between any two of the three loci studied (Table 6). Significant interactions occur also between the *Pgm-1* and *Me-2* loci (Marinković & Ayala, in the press). *Est-5*, *Mdh-2* and *Odh* are all in different chromosomes, but since genotypic interactions among genotypes coding for allozymes appear to be the rule, it seems most likely that they will also exist between loci in the same chromosome, and even between closely linked loci. Linkage disequilibrium between allozyme loci has been demonstrated in natural populations of several species of *Drosophila* (Prakash & Lewontin, 1968, 1971; Roberts & Baker, 1973; Gonzalez-Duarte, Gonzalez Izquiero & Prevosti, 1973; Zouros & Krimbas, 1973).

The reversals of relative fitnesses at various stages of the life-cycle (category 1 above) will not necessarily lead to balanced polymorphism. However, such reversals may result in marginal heterosis (category 2), and thus lead to polymorphic equilibria. Epistatic interactions (category 3) may contribute to the stability of polymorphic equlibria, by mutual reinforcement of the balancing effects of individual loci.

The selective effects just described could occur in a hypothetically uniform and

stable environment. However, natural environments are heterogeneous at any one time, and also vary through time. Some environmental variations, like those associated with the seasons, are cyclical. Environmental heterogeneity is at the basis of two other types of selective effects.

4. *Counteracting selective advantages of the genotypes in different environmental conditions.* Homozygotes for the allele *100* at all three loci (group A, Table 1) have under optimal conditions one of the fastest, and under competitive conditions one of the slowest, rates of development. The opposite situation obtains for flies heterozygous at all three loci (group A × G): they have the fastest rate of development under competitive conditions, but one of the slowest under near-optimal conditions. A similar situation can be seen when the three loci are considered separately (Tables 3–5). At each locus, homozygotes for the most common allele develop significantly faster under optimal conditions, but significantly more slowly under competitive conditions, than heterozygotes. Dobzhansky & Ayala (1973) observed cyclical changes in allozyme frequencies from season to season in natural populations of *D. pseudoobscura*. The observations reported here might conceivably contribute to the oscillations. 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.

5. *Frequency-dependent selection.* The environmental resources are better utilized when a mixture of genotypes is present in a limited environment, than when there is only one genotype. It was found with the *Pgm-1* and *Me-2* loci that the total number of flies produced with a fixed amount of food is much greater when there is a mixture of several genotypes in equal proportions, than when only one genotype is present (Marinković & Ayala, in the press). The average proportion of flies emerging from a given total number of eggs is $50{\cdot}1 \pm 2{\cdot}0\,\%$ when the eggs are a mixture of genotypes, and $41{\cdot}3 \pm 2{\cdot}3\,\%$ when all eggs are of the same genotype. Moreover, the rate of development is significantly faster for the mixture ($16{\cdot}49 \pm 0{\cdot}27$ days) than for the pure culture ($21{\cdot}64 \pm 0{\cdot}43$ days). Presumably the mixed genotypes exploit better the environmental heterogeneity. This is likely to result in an inverse relationship between the frequency of a genotype and its fitness (Ayala & Campbell, 1974), and may lead to stable polymorphic equilibria.

The hypothesis has been suggested that much allelic variation, particularly in genes coding for enzymes and other soluble proteins may be adaptively neutral (Kimura, 1968; King & Jukes, 1969; Kimura & Ohta, 1971). On the contrary, much evidence exists, derived from the study of natural populations as well as from laboratory experiments, suggesting that protein variation is subject to natural selection (see, for example, Prakash & Lewontin, 1968; Allard *et al.*, 1972; Ayala, 1972; Ayala *et al.* 1974). The results reported here support also the hypothesis that protein variation is adaptive. Yet, in a way, our results bring forth an embarrassment of riches. All six fitness components studied are affected by the genetic composition at the three loci taken jointly (Table 1). Genotypic differences at any *one* of the three loci have significant effects on two or more components (Tables 3–5). Present knowledge of the physiological and developmental role of the three

enzymes studied (as, indeed, of most enzymes) is insufficient to ascertain whether allozyme variation is likely to affect the performance of the flies with respect to a given fitness component. But it would seem *a priori* unlikely that a majority of fitness parameters would be *directly* affected to a substantial degree by allelic variation at most enzyme loci.

The effects on fitness of the loci studied might conceivably be due not to the loci themselves, or at least not to them alone, but the loci could be associated in linkage disequilibrium with other loci affecting fitness. This possibility deserves consideration. First, allelic variation might be nonrandomly associated with chromosomal inversions. However, the strains used in our experiment had no chromosomal polymorphisms in any of the chromosomes X, II, and IV, where the three loci are located. The natural population of *D. pseudoobscura* from which the experimental flies were descended is polymorphic for inversions only in the III and in the X chromosomes. But the sole inversion in the X chromosome is SR (Sex Ratio) which was not present in any of our experimental strains.

It might be possible that alleles at the loci studied might accidentally have become nonrandomly associated with allelic variation at other loci having fitness effects. For example, a wild II chromosome carrying allele *100* at the *Odh* locus could by chance have also carried a high fitness allele at some other locus, while a II chromosome carrying allele Odh^{104} could carry a low fitness allele at that other locus. It must be pointed out, however, that at least 21 (and on the average 41·5) different wild chromosomes were extracted carrying each of the allelic variants used in our experiment. Given the large number of strains used in the experiment, accidental associations between the loci studied and other loci must have been largely randomized.

Non-random associations between the loci studied and other loci may have been present in our strains if such non-random associations exist in the natural population from which the experimental flies were obtained. Linkage disequilibrium of enzyme loci with each other, as well as with other loci or with chromosomal inversions has been demonstrated in natural populations of *Drosophila* as well as of other organisms (Prakash & Lewontin, 1968, 1971; Gonzalez-Duarte *et al.* 1973, Roberts & Baker, 1973; Zouros & Krimbas, 1973; Allard *et al.* 1972; Webster, 1973). Genetic coadaptation, i.e. adaptive interactions among loci, is indeed a pervasive, and perhaps universal phenomenon (Dobzhansky, 1951, 1970; Mayr, 1970; Ford, 1971). It seems possible, and indeed likely, that the fitness effects found in our experiment may not be due, at least not only, to the enzyme loci themselves, but also to other loci with which the enzyme loci are associated in linkage disequilibrium. The fitness effects observed may, then, be due to blocks of genes for which the allozymes in our study serve as markers. It should be pointed out, however, that linkage disequilibrium will rapidly decay unless selective interactions exist among the loci involved. Therefore, it seems likely that variation at the enzyme loci studied has effects on adaptation, at least in the form of selective interactions with other loci with which they may be associated in linkage disequilibrium.

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