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'Overcompensation' at an enzyme locus in *Drosophila pseudoobscura**

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SUMMARY

The experiments reported in this paper are primarily addressed to test the hypothesis of overcompensation; i.e. whether polymorphic populations exploit limiting environmental resources better than populations uniform for a single genotype. Overcomposition is an ecological consequence of some models of frequency-dependent selection. Secondarily, the experiments investigate whether overdominance exists at the *Mdh-2* locus in *Drosophila pseudoobscura*.

Two types of experimental populations are established: 'low-variability' populations, in which all flies in a culture are offspring from only two laboratory strains; 'high-variability' populations, in which the flies in a culture are derived from 20 different strains. However, the overall degree of individual heterozygosity is the same in both types of populations. Three kinds of populations with respect to the *Mdh-2* locus are established within each type; two are homozygous for either the *100* or the *112* allele, the third is heterozygous. A fourth kind of population exists among the high-variability populations; namely, populations in which all three *Mdh-2* genotypes are present. The experiments are done at two densities; one quasi-optimal, the other highly competitive.

Populations with high overall levels of genetic variation consistently produce more flies than low-variability populations. The differences are significant at the low, but not at the high, density. Moreover, populations polymorphic for the *Mdh-2* locus generally produce more flies than populations having only one *Mdh-2* genotype. At high density, the *Mdh-2* polymorphic populations have greater productivity than populations with anyone of the three genotypes, and the differences are statistically significant when the polymorphic populations are compared with either one of the two homozygotes or with the average of all three genotypes. In brief, overcompensatory effects – which may account for frequency-dependent selection – are observed in the experiment and may be a common phenomenon in nature.

Populations in which all individuals are heterozygous at the *Mdh-2* locus produce in every case more flies than populations with only

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homozygotes for one or the other allele. The superiority of the heterozygotes is statistically significant for all comparisons at low density, but at high density it is statistically significant for the comparison with the homozygote rarer in nature and only in low variability populations.

INTRODUCTION

Genetic polymorphisms are pervasive in nature. The question of what processes are involved in their maintenance, remains, however, largely unresolved. In recent years, theoretical models as well as experimental evidence have called attention to a potentially major role for frequency-dependent selection (e.g. Clarke & O'Donald, 1964; Harding, Allard & Smeltzer, 1966; Kojima & Tobari, 1969; Anxolabehere & Periquet, 1972; Snyder & Ayala, 1979). If the fitness of alternative genotypes is inversely related to their frequency, a stable polymorphic equilibrium may occur. One important implication is that the genetic load will be, in such case, reduced or altogether eliminated, because at the equilibrium frequency the various genotypes have identical, or similar, relative fitnesses.

One likely ecological explanation for the occurrence of frequency dependent fitnesses is that different genotypes utilize limiting environmental resources, such as food, in different ways. If such is the case, it follows that a mixture of the various genotypes would exploit the resources better than a population uniform for any single genotype – a phenomenon that may be called 'overcompensation'. This paper presents a series of experiments addressed to test this hypothesis, using experimental populations of *Drosophila pseudoobscura*. The existence of overcompensatory effects is tested in two ways. First, by comparing the productivity of cultures with two different levels of genetic polymorphism, although the individual flies possess in both cases similar degrees of heterozygosity. Second, we examine the possible overcompensatory effects of polymorphism at the *Mdh-2* locus, which codes for a malate dehydrogenase enzyme, and is moderately polymorphic in nature. The experiments are also addressed to investigate whether heterozygous superiority – overdominance – exists at this locus. The experiments are made at two densities – one near-optimal, the other highly competitive – to explore the possible density dependence of both, overcomposition and overdominance.

MATERIALS AND METHODS

The strains used in the experiment were derived from 1140 wild females of *Drosophila pseudoobscura* collected in September 1976 in Redwood City, California. The females were individually placed in vials for ovipositing. Several sib-pair matings were made in each of four generations with the progenies of each female in order to obtain homozygosis at the *Mdh-2* locus. In each generation, the genotype of the sib parents was ascertained by electrophoresis and matings having the desired genotypes were chosen to obtain the parents of the following generation. The sib-pair matings were continued for four generations in all lines, even when homozygosis at the *Mdh-2* locus had already been achieved.

A total of 40 strains, each descended from a different wild female, were selected for the experiment. Twenty strains – designated S1, S2, ..., S20 – were homozygous for the *Mdh-2*^{100} ('slow') allele; the other 20 strains – F1, F2, ..., F20– were homozygous for the *Mdh*^{112} ('fast') allele. These strains were maintained throughout the experiment by mass culture in half-pint cultures with a standard agar–cornmeal–molasses medium at a density of about 50 pairs per culture. These strains, as well as the experimental cultures described below, were kept at a constant temperature of $25 \pm 0{\cdot}5$ °C, *c.* 70 % relative humidity, and with a cycle of 12 h light (8 a.m.–8 p.m.) and 12 h darkness.

The experimental cultures were established as follows. Ten newly emerged (0–12 h old) virgin females from one strain were placed with 10 newly emerged males from a different strain in a vial (2×8 cm) with 10 ml of the standard agar–cornmeal–molasses medium. After 5 days, the flies were etherized for 30 s and the females only placed in fresh vials in the combinations and densities described below. After 24 h, the females were transferred without etherization to a fresh vial and allowed to oviposit for 24 h, after which time the females were removed. (Cultures in which any female had died were replaced.) Flies emerging in these cultures were collected and counted from the 17th to the 34th day after egg-laying. Because the mother and father of each of these flies come from two different strains, these flies all carry two different wild homologous chromosomes and, hence, have the same average levels of heterozygosity (except at the *Mdh-2* locus, which was controlled) as wild flies do.

Two types of experimental populations were established that differed in the amount of genetic variation present in a vial with respect to the genetic background (i.e. the whole genome except the *Mdh-2* locus):

(1) *Low-variability populations.* In these populations, all the parental females in a vial came from a single strain and all the males from a second strain. However, all 20 S strains and all 20 F strains were equally represented in different vials. With respect to the *Mdh-2* locus, these low-variability populations were of three kinds. One set produced flies homozygous for the *Mdh-2*^{100} allele; these vials were made by crossing S1 ♀♀ × S2 ♂♂, S2 ♀♀ × S3 ♂♂, etc. The second set produced flies homozygous for the *Mdh-2*^{112} allele; these vials were established by crossing F1 ♀♀ × F2 ♂♂, F2♀♀ × F3 ♂♂, etc. The third set produced flies heterozygous for the *Mdh-2*^{100} and the *Mdh-2*^{112} alleles; these vials were set up by crossing S1 ♀♀ × F1 ♂♂, F2 ♀♀ × S2 ♂♂, etc. Thus, the parental flies in all three sets come from the homozygous stock strains, whereas the progeny flies carry two independent wild genomes, but all flies in a vial have their genomes derived from the same two homozygous strains. The three sets differ from each other by the genotype at the *Mdh-2* locus.

(2) *High-variability populations.* In these populations, 20 different stock strains were represented in each vial. Thus, in the set homozygous for the *100* allele, the females in a given vial came from 10 different S strains (e.g. S1, S2, ..., S10) and were crossed to males from 10 other S strains (S11, S12, ..., S20). Similarly, in the set homozygous for the *112* allele, the females in a given vial came from

10 different F strains (e.g. F1, F2, ..., F10) and were crossed to males from 10 other F strains (F11, F12, ..., F20). In each vial heterozygous for the *Mdh-2* locus, the females came from 10 S or 10 F strains (e.g. S1, S2, ..., S10) that were crossed to males from 10 strains with different *Mdh-2* genotype (e.g. F1, F2, ..., F10). With respect to the *Mdh-2* locus these high-variability populations were of four kinds. Three sets were as in (1): homozygous for allele *100*, homozygous for allele *112*, and heterozygous *100/112*. The fourth set consisted of all three genotypes in equal proportions, i.e. one third females *100* crossed to males *100*, one third females *112* crossed to males *112*, and one third females *100* (or *112*) crossed to males *112* (*100*). (However, in the cultures with a density of 10 females – see below – there were three crosses of each of two kinds and four of the third kind, but the kind represented in excess was evenly distributed among replicates). Therefore, as in the low-variability populations, the parental flies in these high-variability populations came from the homozygous stock strains, whereas the progeny flies carried two independent wild genomes. However, in the high-variability populations, 20 different stock strains – rather than only two – were represented in each vial. Hence, the populations were much more polymorphic, at loci other than the *Mdh-2* locus, in the high-variability vials than in the low-variability vials. With respect to the *Mdh-2* locus, three of the high-variability sets were the same as the low-variability sets (two homozygous sets and one heterozygous set); in addition, there was a fourth set which was polymorphic – all three *Mdh-2* genotypes were present in each vial.

The experiments were conducted at two densities: (*a*) *low-density*, with 10 females per vial, and (*b*) *high-density*, with 30 females per vial. Each genetic combination was replicated 40 times at each density; that is, there were a total of 120 low-variability and 160 high-variability vials at each density.

The results were analyzed using two-way and three-way analyses of variance (Sokal & Rohlf, 1969) and paired-mean comparisons.

RESULTS

The *Mdh-2* locus codes for a malate-dehydrogenase (E.C. 1.1.1.37) enzyme involved in the citric acid cycle. In *Drosophila pseudoobscura*, *Mdh-2* is located on the fourth chromosome, in which no inversion polymorphisms are known in nature. Several alleles are found in natural populations at the *Mdh-2* locus: $Mdh\text{-}2^{100}$, with a frequency about 0·95; $Mdh\text{-}2^{112}$ with a frequency about 0·03, and several rare alleles, each with a frequency below 0·01. Only the two more common alleles, *100* ('slow') and *112* ('fast') are used in the present experiment.

The experimental results are summarized in Table 1. The table gives the mean and standard errors for the number of flies emerged in each vial, according to genotype and density. The productivity is generally greater at the higher than at the lower density ($38{\cdot}9 \pm 0{\cdot}8$ versus $35{\cdot}6 \pm 0{\cdot}9$), but the increase in productivity is relatively small compared to the three-fold increase in the number of parents. Competition among the larvae is, indeed, intense in the high-density vials.

Table 1. *Means and standard errors of productivity in populations with different constitutions at the* Mdh-2 *locus, different amounts of variability in the background, and different densities. Low density = 10 ♀♀/vial; high density = 30 ♀♀/vial*

Genotype		Low density		High density	
Background	*Mdh-2*	*N*	Mean	*N*	Mean
Low variability	*112/112*	40	23·38 ± 1·92	40	30·07 ± 1·82
	100/100	40	27·90 ± 1·99	40	38·07 ± 2·12
	100/112	40	34·77 ± 2·26	40	39·87 ± 2·86
	Mean	120	28·68 ± 1·25	120	36·01 ± 1·38
High variability	*112/112*	40	36·75 ± 2·14	40	37·05 ± 1·76
	100/100	40	38·22 ± 2·11	40	40·25 ± 1·49
	100/112	40	44·42 ± 2·35	40	41·07 ± 1·95
	Mean	120	39·80 ± 1·29	120	39·46 ± 1·01
	Mixed	40	43·62 ± 2·16	40	45·65 ± 2·17
Combined	Overall mean	280	35·58 ± 0·91	280	38·86 ± 0·82

Table 2. *3-way analysis of variance for the productivity values given in Table 1 ('mixed' populations are not included)*

Source	MS	D.F.	F
Density (D)	1,463·01	1	8·37**
Genetic background (B)	6,365·63	1	36·42***
Mdh Genotype (G)	2,707·90	2	15·49***
D × B	1,763·33	1	10·09
D × G	273·01	2	1·56
B × G	257·66	2	1·47
D × B × G	12·26	2	0·07
Error	174·76	468	
Total		479	

** Statistically significant $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Table 1 also shows higher productivity in populations having high background-variability than in those having low background-variability. There is also a consistent gradual increase in productivity, within each background and density, from the homozygotes rare in nature (*112/112*) to the common homozygotes (*100/100*) to the heterozygotes (*100/112*); the 'mixed' populations that contain all three *Mdh-2* genotypes generally have higher productivity than any of the single-genotype populations.

The results of a 3-way analysis of variance are given in Table 2. The mixed populations are not included in this ANOVA because such populations were not set up with a low-variability background. Table 2 shows significant heterogeneity for each one of the three factors: density, genetic background, and *Mdh-2* genotype. The only statistically significant interaction in Table 2 is that between density and the genetic background. The variation due to density is due to the higher productivity of the cultures with 30 female parents. But their

relative small increase compared to the low-density cultures suggests that larval competition is much more intense at the higher density. With respect to genetic background, the highly significant effect manifest in Table 2 is due to the greater productivity of the populations with high levels of genetic variation within a vial.

The effects of the *Mdh-2* genotype are examined in Table 3 by means of paired *t*-test comparisons. At the lower density, the heterozygous cultures produce a significantly greater number of flies than either one of the two homozygotes; in the populations with low-variability background as well as in those with high-variability background. At the high density, the heterozygous cultures again produce greater numbers of flies than either homozygote (see Table 1), but the differences are statistically significant only for the comparison with the rare homozygote (*112/112*) and only when the background has low variability. Cultures with the homozygous genotype more common in nature (*100/100*) produce in every case more flies than those with the rarer homozygous genotype (Table 1),

Table 3. *Paired* t*-test values* (D.F. = *78*) *for comparisons of populations with different genotype at the* Mdh-2 *locus*

	Low density		High density	
Populations compared	Low variability	High variability	Low variability	High variability
112/112–100/100	−1·64	−0·49	−2·86	−1·39
112/112–100/112	−3·85***	−2·41**	−2·88**	−1·53
100/100–100/112	−2·28*	−1·97*	−0·51	−0·34

* Statistically significant $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Table 4. *Analyses of variance for the populations with high background variability* (*'mixed' populations included*)

Source	MS	D.F.	F
(*a*) 2-way ANOVA for populations with high variability			
Density (D)	5·00	1	0·03
Mdh Genotype (G)	175·77	3	1·06
D × G	916·76	3	5·54**
Error	165·39	312	
Total		319	
(*b*) ANOVA for populations at low density			
Mdh genotype	588·62	3	3·06*
Error	192·18	156	
Total		159	
(*c*) ANOVA for populations at high density			
Mdh Genotype	503·91	3	3·64*
Error	138·60	156	
Total		159	

* Statistically significant $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

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but the differences are significant only at high density when the background has low variability.

Populations with all three *Mdh-2* genotypes mixed in the same vial were made at both densities but only in the set with high variability in the genetic background (indeed, they could not be made in the low variability cultures, because only two strains were used for each such culture). The results of a 2-way analysis of variance for these high-variability populations are given in Table 4*a*. Neither density nor the *Mdh-2* genotype show statistically significant effects. But the interaction between density and genotype is statistically significant and, therefore, the effects of the *Mdh-2* genotype are separately analyzed for high and for low density by means of one-way analyses of variance (Tables 4*b* and 4*c*). At either density, the genotype at the *Mdh-2* locus has statistically significant effects. These effects are further examined by paired *t*-test comparisons in Table 5.

Table 5. *Paired* t*-test values for the difference in productivity between each of the various populations homogeneous at the* Mdh-2 *locus and the* '*mixed*' *population*

Population compared	Degrees of freedom	Low density	High density
112/112	78	−2·26*	−3·07**
100/100	78	−1·79	−2·05*
100/112	78	0·25	−1·56
Mean	158	−1·52	−2·88**

* Statistically significant $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

At the low density, at which competition among larvae is weak, the mixed population has significantly higher productivity than the rare homozygote, but is not significantly different from either one of the two other genotypes or from the mean of all three. However, at the high density the differences between the mixed population (which always yields a greater number of flies) and either one of the two homozygotes are statistically significant. The greater amount of polymorphism present in the mixed populations results in significantly greater productivity also when the mixed populations are compared with the average of all three genotypes. These effects of the *Mdh-2* locus occur in spite of the high level of genetic variation present in the background of all these populations.

DISCUSSION

In this paper we examine two processes that may contribute to the maintenance of genetic polymorphisms: overdominance – when the heterozygotes have higher fitness than the corresponding homozygotes – and negative frequency-dependent selection – when the fitness of alternative genotypes is negatively related to their frequency.

Evidence of frequency-dependent selection has been gradually accumulating in recent years with respect to a variety of fitness components, such as male

mating ability (e.g. Spiess, 1957; Petit & Ehrman, 1969) and viability (e.g. Yarbrough & Kojima, 1967; Snyder & Ayala, 1979). We are particularly interested in the implications of frequency-dependent selection with respect to viability. A negative correlation between the frequency of genotypes and their fitness can be accounted for, in ecological terms, if alternative genotypes exploit different environmental resources, or exploit them differentially. Then, as one genotype becomes more common, the environmental resources that it exploits best become more saturated and the genotype competes more and more for the resources that are best exploited by the alternative genotype(s). As a consequence, the relative fitness of the genotype that is becoming increasingly common gradually decreases. If this explanation is correct, it follows that the environmental resources will be better exploited by a combination of several genotypes than by only one genotype. Secondary interactions (e.g. catabolites produced by one genotype being harmful or beneficial to other genotypes) may intervene, but it should nevertheless be generally the case, when there is competition for resources, that the occurrence of negative frequency dependence implies a better exploitation of the resources by a genotype mix than by a single genotype (Levins, 1965).

The main hypothesis tested in the present paper is this implication of negative frequency dependence: whether a mixture of genotypes exploits better the environment than a single genotype. This hypothesis is tested in two ways: (1) with respect to the overall genotype with the exclusion of the *Mdh-2* locus; and (2) with respect to the *Mdh-2* locus. It is this second test that is of particular interest as a possible explanation of the polymorphism observed at this locus and of the pervasive electrophoretic polymorphisms observed in natural populations. A second hypothesis tested is overdominance: whether the heterozygotes for the *Mdh-2* locus exhibit higher fitness than the homozygotes.

The results show that density has a statistically significant effect on the number of flies emerged from the cultures (Table 2). But the overall mean number of emerged flies is only about 10% greater in the high-density than in the low-density cultures.

More interesting for our purposes are the effects of the levels of polymorphism in the genotype as a whole (with the exclusion of the *Mdh-2* locus). The effect of the genetic background is statistically very highly significant (Table 2), with high background-variability resulting in a higher number of flies as predicted by the hypothesis. The differences are, however, considerably larger at the low density (39·80 vs. 28·68 flies per culture) than at the high density (39·46 vs. 36·01 flies).

The main objective of the experiments was to test whether an increase in the level of polymorphism at the *Mdh-2* locus would result in an increase in the number of flies emerged in a culture. This would indicate that the *Mdh-2* genotypes exploit the environmental resources in different ways and, hence, that frequency-dependent selection may act at this locus. The test was made only in cultures with high genetic variability in the background, but such condition more nearly approximates the natural situation. Indeed, in natural populations,

polymorphism in the genotype as a whole is high. In the high-variability cultures, the competing larvae are all derived from crosses between different stocks and the level of background genetic variability is similar to that occurring in nature.

Tables 4b and 4c show that the *Mdh-2* genotype has a statistically significant effect at low as well as at high density. At low density, the cultures with all three genotypes combined produce significantly more flies only when compared with the cultures having flies homozygous for the genotype (*112/112*) rarer in nature. However, when competition is intense, the mixed-genotype cultures produce more flies than any one of the other cultures, and the differences are statistically significant for the comparisons with either homozygote or with the mean of all three genotypes (Table 5). These results corroborate the hypothesis proposing that the *Mdh-2* genotypes exploit the environmental resources in different ways, and that the environment is better exploited by populations with all three genotypes present than by populations fixed for one or the other allele. This, in turn, suggests that the *Mdh-2* locus may be subject to frequency-dependent selection, a result confirmed in separate experiments made with the same strains (Tošić & Ayala, 1980).

Antonovics (1978) has emphasized the importance of intraspecific competitive interactions in maintaining polymorphisms. Stable polymorphisms become possible if individuals of one genotype are more inhibited by individuals with the same genotype than by individuals with other genotypes. Evidence of overcompensatory effects exists for plants such as *Linum* (Harper, 1967), barley and wheat (Allard & Adams, 1969). In *Drosophila* overcompensation has been demonstrated for populations polymorphic for chromosomal arrangements (Dobzhansky & Pavlovsky, 1961) and for populations obtained by mixing the gene pools from different local populations (Ayala, 1965). Models of overcompensatory effects leading to the maintenance of single-gene polymorphisms have been proposed and tested by computer simulations (Schultz & Usanis, 1969; Antonovics, 1978).

The second hypothesis tested in our experiments is overdominance. The heterozygotes have higher fitness than either homozygote at low density, when the genetic background variability is high as well as when it is low (Tables 1 and 3). At high density, the heterozygotes have higher productivity than the homozygotes, but the differences with the most common homozygote (*100/100*) are small and not statistically significant. Changes in the relative fitness values of various genotypes under different environmental conditions or when different fitness components are considered have been observed at the *Mdh-2* locus as well as at other enzyme loci by Marinković & Ayala (1975*a*, *b*). Generally, the fitness of the heterozygotes is as good as, or better than, that of the 'best' homozygote; but which one is the best homozygote depends on the environmental condition or fitness component being considered. The net result may be 'marginal overdominance' (Wallace, 1968) – when all fitness components are taken into account and/or when the environmental conditions oscillate, the net fitness of the heterozygote may be superior to either homozygote. Our experiment suggests that if the density of a population in nature oscillates between high and low density

in different subenvironments or at different times, net overdominance would result. Overdominance is, of course, present at low density. Seasonal density oscillations in natural populations of *D. pseudoobscura* have been proposed as a possible explanation for observed oscillations in the frequency of some allozymes (Dobzhansky & Ayala, 1973).

The question arises whether the observed overcompensatory and overdominance effects may be attributed to the *Mdh-2* locus or to other locus (or loci) associated with it in linkage disequilibrium. The possibility of the *Mdh-2* locus being nonrandomly associated with chromosomal inversions can be rejected, because no chromosomal polymorphisms exist in the fourth chromosome in the strains of *D. pseudoobscura* used for the experiment. An accidental nonradnom association between the *Mdh-2* locus and other loci is minimized by the sampling of 20 independent chromosomes from the natural population for each allele. This insures that more than 95% of the genetic variation present in the natural population is represented in the experimental populations (Nei, Maruyama & Chakraborty, 1975). However, nonrandom association with alleles at other loci closely linked to the *Mdh-2* locus, that might be, in part or in *toto*, responsible for the effects attributed to the *Mdh-2* locus cannot be rejected. But if such nonrandom association is present in our experiments it must also be present in the natural population. And so long as such association remains in the natural population, the effects herein observed would also occur in nature and thus contribute there to the maintenance of the *Mdh-2* polymorphism. The possibility that such natural association, if it exists, is only transient is minimized by the fact that the allele frequencies at the *Mdh-2* locus are nearly the same in all natural populations of *D. pseudoobscura* studied.

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