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MDH-Polymorphism in *Drosophila subobscura*: I. Selection and Hitch-hiking in Laboratory Populations

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Summary. The interrelation of genic polymorphism at the Malate dehydrogenase (Mdh)-locus and chromosomal polymorphism for superimposed gene arrangements was studied in 5 experimental populations of *Drosophila subobscura*. It could be shown that chromosomal polymorphism is maintained by balancing selection in favour of the heterozygotes. In contrast, selection at the Mdh-locus itself seems to be of minor importance. The changes of frequency observed for the Mdh-alleles are most probably due to hitch-hiking on the gene blocks enclosed by the chromosomal structures or to selection for tightly linked genes rather than to fitness differences between Mdh-genotypes. The results may be considered as a model for the situation found in natural populations of *D. subobscura*.

Key words: Allozyme polymorphism – Inversion polymorphism – Selection – Linkage – Hitch-hiking – *Drosophila subobscura*

Introduction

The scarcity of direct experimental evidence for the operation of selective forces on allozyme loci has to be considered as the main reason for the persistance of the controversy between adherents of the neutral theory of evolution and orthodox neo-Darwinian selectionists. Since the late sixties, when the dispute was brought up by Kimura (1968) and King and Jukes (1969), both sides have tried equally hard to provide experimental data supporting their theories. In this competition, the selectionist party has to carry a severe handicap. In order to prove selection at a specific gene locus under study it is not sufficient to demonstrate selective response. The main problem is to exclude any influence of the genetic background since otherwise the results are exposed to the critical objection that the observed selection effect has to be ascribed to associated loci or chromosomal structures (see Jones and Yamazaki 1974). On the other hand, the position of the neutralist party is much more comfortable. Any result failing to prove unambiguously selection at a single structural gene is claimed as a confirmation of the neutral theory.

In order to elucidate the interrelations between selection at individual loci and linkage disequilibria with adjacent genes, two investigations on the Mdh-polymorphism of *Drosophila subobscura* were carried out which describe the situation in wild populations (Pinsker and Sperlich, in prep.) as well as in the laboratory (present paper). At first sight the Mdh-locus seems to be rather unsuitable for such studies. Allozyme investigations of samples from several European wild populations have revealed only very low variation at this locus: e.g. Finland, Southern Scandinavia, France, Eastern Alps (Saura et al. 1973); Greece, Crete (Zouros et al. 1974); Germany (Pinsker et al. 1978); Yugoslavia (Marinkovć et al. 1978); Switzerland, Italy (Pinsker and Sperlich 1979); Scotland, U.S.S.R. (Sperlich et al. 1981). Two southern populations from Sicily and Tunesia, however, proved rather polymorphic for Mdh-allozymes (Pinsker and Sperlich 1979). The same was recently shown for at least some populations from the Canary Islands (Cabrera et al. 1980). In all these populations the otherwise rare allele Mdh^{105} was found with a frequency of almost 8%. This remarkable increase is paralleled with a higher frequency of the chromosomal structure U_{1+2+8}. Simultaneous analysis of both genic and chromosomal polymorphism revealed non-random associations between Mdh-alleles and inversions of chromosome U where the Mdh-gene is located. The associations are not restricted to those populations with high frequencies of Mdh^{105} but also present in other populations where Mdh-polymorphism is rather low (Sperlich and Pinsker 1980). In the wild populations studied so far, linkage disequilibrium is not absolute although the

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gene arrangements differ significantly with respect to the relative frequencies of Mdh-alleles. A complete association, however, was detected in a laboratory strain originated with a population sample from Tunesia. In this laboratory population an excess of Mdh-heterozygotes was observed among adult flies, whereas in larvae genotypes were distributed according to the Hardy-Weinberg law (Sperlich et al. 1976). Summing up all these findings it seemed worthwhile to examine in more detail the selective forces putatively involved in maintaining the polymorphisms of Mdh and the surrounding chromosomal structures as well as the linkage disequilibria. For this purpose five experimental populations were started with different initial frequencies for Mdh-allele/gene-arrangement combinations. The effects of selection were recorded by checking electrophoretically the allozyme constitution of the strains over a number of generations.

Material and Methods

Strains

Two wild-type strains derived from Tunesia were available in our laboratory:

Tunesia I (polymorphic for the combinations Mdh^{96}/U_{1+2} and Mdh^{105}/U_{1+2+8}) and

Tunesia II (homozygous for Mdh^{96}/U_{1+2+8} which is the most frequent combination in Tunesian populations).

Both strains originate from mass cultures of wild population samples.

Experimental population A was founded by transferring the polymorphic strain Tunesia I from bottle cultures to a population cage. For the other populations (B, C, D, and E) stocks homozygous for the different allele/gene-arrangement combinations had to be established. In order to neutralize the genetic background the two strains Tunesia I and Tunesia II were crossed together and then kept polymorphic for two more generations thus allowing genetic recombination. Thereafter single pair crossings were performed for obtaining three lines homozygous for Mdh^{96}/U_{1+2}, Mdh^{96}/U_{1+2+8}, and Mdh^{105}/U_{1+2+8} respectively. During this crossing procedure none of the breeding lines had to pass a bottleneck smaller than 20 single pair cultures per generation. Therefore, inbreeding and founder effects were thought to be of minor influence on the results obtained in the populations derived from these strains. – The populations B, C, D, and E were started with 500 virgin females and 500 males each. The initial frequencies of the different allele/gene-arrangement combinations were as follows:

population		
A	78% Mdh^{96}/U_{1+2}	22% Mdh^{105}/U_{1+2+8}
B	90% Mdh^{96}/U_{1+2}	10% Mdh^{105}/U_{1+2+8}
C	10% Mdh^{96}/U_{1+2}	90% Mdh^{105}/U_{1+2+8}
D	90% Mdh^{96}/U_{1+2+8}	10% Mdh^{105}/U_{1+2+8}
E	10% Mdh^{96}/U_{1+2+8}	90% Mdh^{105}/U_{1+2+8}

The frequencies given for population A were determined before the transfer to the population cage. Populations B, C, D, and E were set up mixing adult flies from the respective homozygous lines according to the desired ratio of genotypes. All 5 populations were kept in population cages thus ensuring random mating. Fresh medium was added weekly. Effective population size was estimated at approximately 3000 individuals. Egg samples were taken at intervals of 28 days, which can be considered as the average generation length at 18°C. In populations A, B, and C temperature was changed to 14°C after generation 11, thus extending the generation length (and the sample intervals) to 36 days.

Electrophoretic Analysis

Electrophoresis was carried out on whole fly homogenates on horizontal starch gels (tris-citrate pH 7.1). Because of the high numbers of individuals to be studied, the usual method had to be modified. Each gel (20 cm × 25 cm) was set up with 4 startlines instead of one, each startline containing the homogenates of 40 flies absorbed onto pieces of filter paper (10 mm × 2.5 mm). Malate dehydrogenase (Mdh) was stained according to the method given by Ayala et al. (1972).

Results

The frequencies of the three Mdh-genotypes (96/96, 96/105, and 105/105) in the five populations are summarized in Table 1. Since the offspring of the first egg samples taken 28 days after the start actually represents the second generation, no data are available for generation 1. Population A was started two generations earlier than the rest. Therefore, the data given for generation zero represent the results of the first egg sample.

The allele frequencies in population A, B, and C, where the Mdh-alleles were enclosed in different gene arrangements, are depicted in Fig. 1. In population A the proportion of allele 96 (the predominant allele in natural populations) remained rather constant (at about 68%) throughout the 18°C experiment, indicating a stable equilibrium. Compared to the Tunesia I strain kept in glass bottles the frequency of Mdh^{96} at equilibrium shifted slightly from 78% to 68% in favour of the allele Mdh^{105}. This might be explained as a selective response to the altered culture conditions after the transfer to the population cage.

In both population B and population C selection against the more frequent allele was observed during the first five generations. In population B the frequency of allele 96 decreased from 90% to an equilibrium value (averaged from generation 5 to 11) of 61%. In contrast allele 96 increased its frequency from 10% to 50% (averaged from generation 5 to 11) in population C.

According to these results all three populations arrived at stable equilibria within a few generations. Moreover, the equilibrium frequencies are rather alike in all three populations (although the differences are statistically significant). The mode of selection recorded especially for population B and population C strongly suggests overdominance, an interpretation supported by the heterozygote advantage previously proved for the Tunesia I strain (Sperlich et al. 1976). Assuming overdominance, the relative magnitudes of the two selection coefficients for the

Table 1. Frequencies of the 3 Mdh-genotypes in 5 experimental populations (A, B, C, D, E). Population A was started at equilibrium. Populations B, C, D, and E were started with homozygotes only. Populations A, B, and C were kept at colder temperature (14°C) after generation 11. Populations D and E remained at 18°C throughout the experiment. (S = Mdh^{96}; F = Mdh^{105}; n = 300 flies/generation for each population)

		A			B			C			D			E		
Genotypes		S/S	S/F	F/F	S/S	S/F	F/F	S/S	S/F	F/F	S/S	S/F	F/F	S/S	S/F	F/F
Generation	0	.389	.505	.105	.900	—	.100	.100	—	.900	.900	—	.100	.100	—	.900
	1	.443	.443	.113	—	—	—	—	—	—	—	—	—	—	—	—
	2	.443	.463	.093	.727	.233	.040	.157	.413	.430	.427	.480	.093	.007	.097	.897
	3	.443	.420	.137	.587	.360	.053	.120	.563	.317	.427	.510	.063	.017	.110	.873
	4	.444	.447	.109	.477	.444	.079	.132	.519	.349	.312	.525	.163	.000	.080	.920
	5	.587	.363	.050	.307	.500	.193	.277	.577	.147	—	—	—	—	—	—
	6	.430	.523	.047	.423	.473	.103	.193	.633	.173	—	—	—	—	—	—
	7	.447	.503	.050	.410	.460	.130	.177	.477	.347	—	—	—	—	—	—
	8	.403	.417	.180	.320	.507	.173	.230	.473	.297	—	—	—	—	—	—
	9	.473	.460	.067	.413	.513	.073	.187	.560	.253	—	—	—	—	—	—
	10	.447	.460	.093	.303	.437	.260	.297	.567	.137	—	—	—	—	—	—
18°C	11	.450	.480	.070	.370	.520	.110	.237	.580	.183	.190	.530	.280	.017	.203	.780
14°C	12	.327	.567	.107	.403	.537	.060	.250	.543	.207	—	—	—	—	—	—
	13	.483	.453	.063	.373	.507	.120	.177	.477	.347	—	—	—	—	—	—
	14	.330	.520	.150	.377	.433	.190	.170	.603	.227	—	—	—	—	—	—
	15	.297	.523	.180	.230	.547	.223	.140	.510	.350	.043	.530	.427	.003	.243	.753
	16	.333	.497	.170	.377	.457	.167	.223	.447	.330	—	—	—	—	—	—
	17	.317	.543	.140	.327	.473	.200	.110	.527	.363	—	—	—	—	—	—
	18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	20	—	—	—	—	—	—	—	—	—	.055	.346	.599	.013	.260	.727

homozygotes can be deduced from the allele frequencies at equilibrium. Furthermore, knowing the ratio of the two coefficients, the actual values can be estimated from the rate of change of the allele frequencies on the way to equilibrium. This was done by means of a Chi-sqare test for goodness of fit, adapting a theoretical selection curve to the experimental data (Fig. 2). The selection coefficients obtained this way are rather high, implying that heterozygotes are more than twice as fit as each of the homozygotes (Table 2). Comparing the observed frequen-

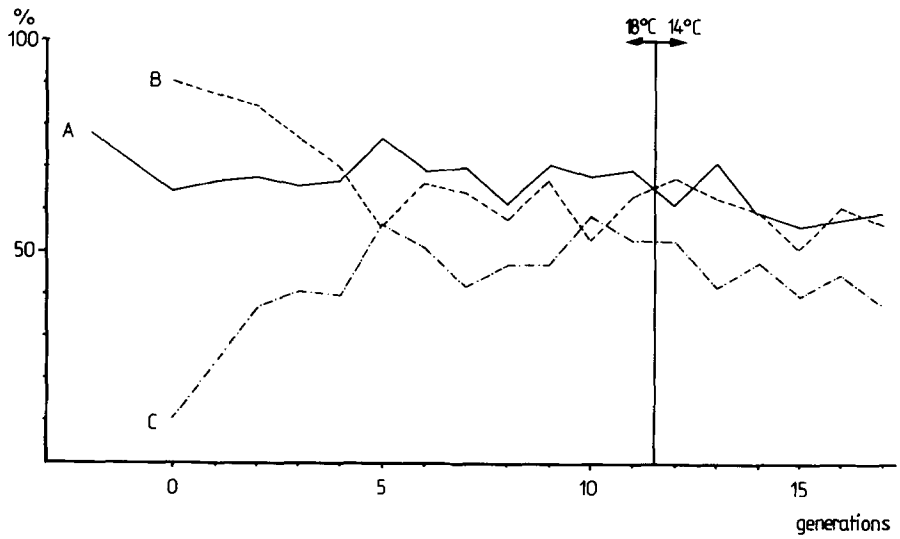

Fig. 1. Frequencies of the allele Mdh^{96} in population A, B, and C. Population A was transferred from culture bottles to the population cage 2 generations before populations B and C were started. The initial frequency corresponds to the frequency determined for the bottle cultures. After generation 11 the temperature was changed from 18°C to 14°C

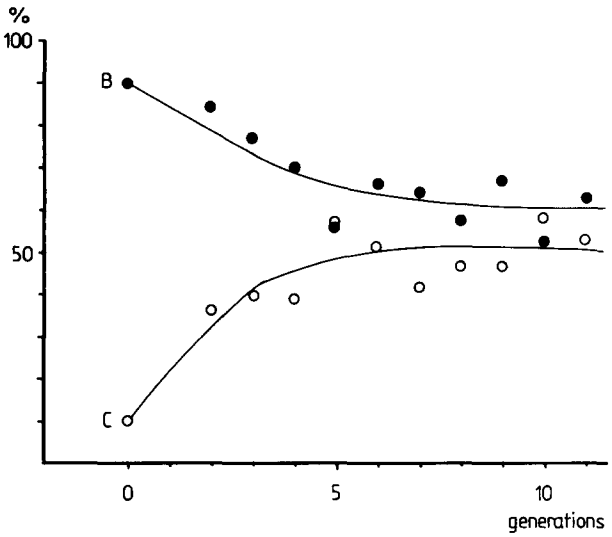

Fig. 2. Best fitting theoretical selection curves for population B and C. Observed allele frequencies (Mdh^{96}) are given as black circles (B) and open circles (C)

Table 3. Average excess of heterozygotes (in % of the expected frequency)

Population	A	B	C	Temperature
Generations	1–11	5–11	5–11	18°C
	5.3%	5.0%	11.8%	
Generations	15–17	15–17	15–17	14°C
	6.8%	0.4%	3.2%	

Table 4. Relative viabilities of Mdh-genotypes calculated from the observed excess of heterozygotes at equilibrium

Population	A	B	C
Generations	1–11	5–11	5–11
Mdh 96/96	0.923	0.922	0.791
Mdh 96/105	1.000	1.000	1.000
Mdh 105/105	0.835	0.879	0.789

Table 2. Fitness values for Mdh-genotypes calculated from the observed frequency changes. ($\bar{q}$ = frequency of the allele Mdh^{96} at equilibrium)

Population	A	B	C	D	E
18°C					
Mdh 96/96	----	0.52	0.36	0.54	0.93
Mdh 96/105	----	1.00	1.00	1.00	1.00
Mdh 105/105	----	0.28	0.33	0.83	0.97
		$\bar{q}$ = .60	$\bar{q}$ = .51	$\bar{q}$ = .27	$\bar{q}$ = .29
14°C					
Mdh 96/96	0.56	0.76	0.46	----	----
Mdh 96/105	1.00	1.00	1.00	----	----
Mdh 105/105	0.40	0.69	0.63	----	----
	$\bar{q}$ = .58	$\bar{q}$ = .56	$\bar{q}$ = .41		

cies of the genotypes (Table 1) with those according to Hardy-Weinberg distribution calculated from the observed allele frequencies, a distinct excess of heterozygotes becomes apparent in populations A, B, and C. Table 3 shows the average excess of heterozygotes for each population based on the data of only those generations where the populations were in equilibrium. Because of random fluctuations of the allele frequencies, the excess of heterozygotes was determined for each generation separately and expressed as the percentage of expected heterozygotes. From the heterozygote excess averaged over several generations relative viabilities of the genotypes can be estimated. As it can be seen from Table 4, the differences in viability between homozygotes and heterozygotes measure about 10%, which is quite similar to the values previously found for the Tunesia I strain, comparing the genotype distributions among larvae and adults (Sperlich et al. 1976). Thus, viability accounts only for a minor part of the fitness differences which gave rise to the strong selection in population B and C, and it can be concluded that reproductive fitness components (e.g. fertility) are much more important.

After generation 11, populations A, B, and C were exposed to lower temperature (14°C). As a result parallel changes in allele frequency at the expense of Mdh^{96} could be observed. The selective forces underlying these changes are again rather high (Table 2). The superiority of the heterozygotes with respect to viability persists but is not high enough for explaining the fitness differences (Table 3). The immediate selective response indicates that the

equilibrium of the heterotic system marked by the Mdh-alleles, although balanced by strong selection, is sensitive to alterations of the environment.

In population D and E both alleles Mdh^{96} and Mdh^{105} were combined with the gene arrangement U_{1+2+8}. In population D, allele 96 declined from a frequency of 90% to 23% after 20 generations. In population E the frequency of the same allele increased slightly from 10% to 15% (Table 1 and Fig. 3). Hence the selection process in the two populations can be interpreted in such a way that both populations are approaching an equilibrium at a frequency of about 28% for allele 96. Adapting theoretical selection curves to the data (Fig. 3), the best fits are obtained by assuming overdominance. This explanation, however, is not as convincing as for populations A, B, and C. For population E the calculated selective disadvantage of the homozygotes (Table 2) seems to be negligible and the frequency changes of the alleles can be regarded as random fluctuations as well. In population D the 96/96 homozygotes are obviously selected against. According to Table 2 the 105/105 homozygotes have also a lower fitness than the heterozygotes but the difference is less pronounced. Although excess of heterozygotes was observed in some generations the mode of selection cannot be decided unambiguously for this population.

Another interpretation of the data from population D and E could be the assumption that the Mdh-allele 96 was linked to a detrimental gene at the beginning of the selection experiment. The decrease of the frequency of allele 96 in both populations during generations 2, 3, and 4 seems to confirm this hypothesis. Accordingly the reversal of the selection process in population E and the slowing down of selection in population D might be due to crossing over and successive separation of the Mdh^{96} allele from the disadvantageous neighbouring gene. Whatever the case might be, it becomes evident that in population D and E where the two Mdh-alleles were on the same gene arrangement selection run completely different than in population B and C where the Mdh-alleles were enclosed in different chromosomal structures.

Discussion

The main intention of the present study was to obtain additional information about the selective forces maintaining the polymorphism at the Mdh-locus in the laboratory populations as well as in wild populations of *D. subobscura*. According to the experimental data obtained from the 5 populations the problem seems to be rather complex. The different outcome in the two groups of populations (A, B, C vs. D, E) strongly indicates the importance of linkage and genetic background. In populations A, B, and C, where the Mdh-alleles were associated with different chromosomal structures, a stable equilibrium was achieved which can be best explained by balancing selection. Without knowing about the chromosomal structures, the most plausible interpretation of this result would be the assumption of different fitness for the Mdh-genotypes, ascribing the highest value to the heterozygotes. Thereupon one could be induced to speculate over the biochemical reasons for the advantage of the heterozygotes. The data from population D and E, however, contradict this explanation. Although the allele frequencies at the Mdh-locus changed also in these populations, the fitness values for the Mdh-genotypes do not resemble the situation in populations A, B, and C. Furthermore, the strong selection in favour of the heterozygotes dis-

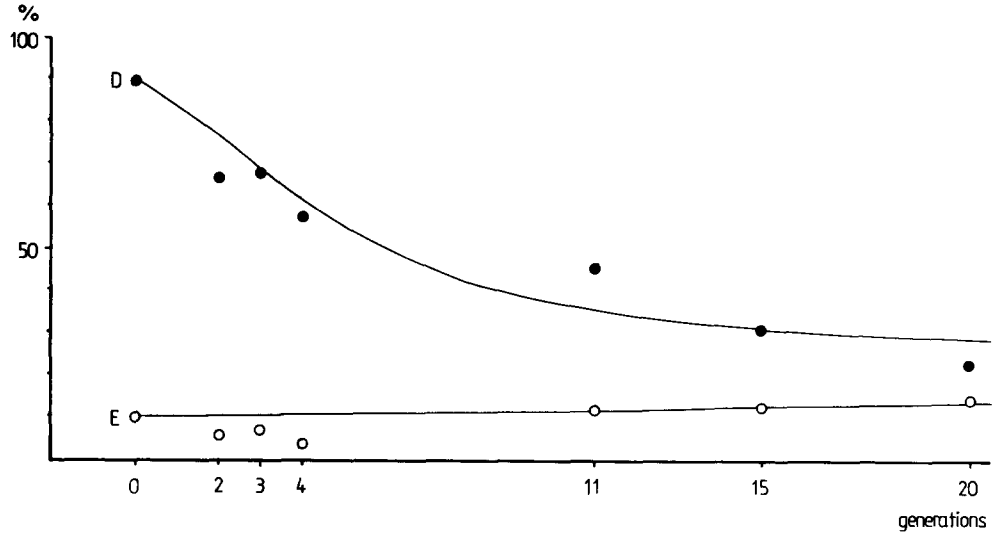

Fig. 3. Best fitting theoretical selection curves for population D and E. Observed allele frequencies (Mdh^{96}) are given as black circles (D) and open circles (E)

appeared. Hence it has to be concluded that not the Mdh-locus itself but the superimposed gene arrangements gave rise to the heterotic effect. Selection probably did not act at the single gene level but on the entire gene blocks held together by the inversions. Whether the Mdh-alleles contribute to some extent to the selective value of the gene-system in which they are included remains an open question.

In populations D en E the gene blocks around the Mdh-alleles were broken down by crossing over. As a consequence the Mdh-genotypes were provided with completely different fitness values. Since the genetic background was widely neutralized by recombination one would expect these values to represent the actual effect of the Mdh-locus on fitness. Accordingly, Mdh^{96} appears as a detrimental allele which is obviously selected against. This, however, is hard to believe since Mdh^{96} was found to be the predominant allele in all natural populations studied so far. Yet, as has been discussed above, even after destroying the absolute associations, disequilibria between closely linked genes will persist for quite a long period in spite of free crossing over. Therefore, it can be assumed that again the fitness values attributing to the Mdh-genotypes are influenced by neighbouring genes although not as strikingly as in those cases where the genes were held together by inversions.

Are these laboratory experiments suitable for understanding the situation in those wild populations where Mdh proved polymorphic? Although the same associations between Mdh-alleles and inversions occur in nature no indication for balancing selection has been found so far. The frequencies of the Mdh-alleles at equilibrium in populations A, B, and C are not consistent with the allele frequencies in wild populations. Yet, it has been demonstrated that the equilibrium is strongly influenced by both the environment and the genetic background. The laboratory conditions certainly do not resemble the natural environment of *D. subobscura*. In addition, establishing laboratory stocks is always accompanied by a reduction of the genetic variation of the sample giving rise to founder effects. However, the proof of strong selection regulating the frequencies of gene arrangements probably indicates that similar selection processes take place in natural populations too and play at least some role in the maintenance of the chromosomal polymorphism of *D. subobscura*. On the other hand, the hitch-hiking of alleles on gene arrangements could serve as an explanation for allelic differences between populations and even for clinal variation. Thus, the laboratory experiments can be considered as simplified models for the complicated interference of chromosomal and genic polymorphism in wild populations of *D. subobscura*.

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