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Isoenzyme Frequencies in Long-term Selection Lines of *Drosophila melanogaster* III. Isoenzyme Frequencies of the Esterases (Est) and Larval Alkaline Phosphatases (Aph) in Selection Lines for Short and Long Developmental Rate*

Hans-J. Muhs

Forstbotanisches Institut der Albert-Ludwigs-Universität, Freiburg, and Institut für Forstgenetik und Forstpflanzenzüchtung der Bundesforschungsanstalt für Forst- und Holzwirtschaft, Schmalenbeck (BRD)

Summary. 1) A heterogeneous initial population which was founded by a double cross of four laboratory stocks was selected for developmental rates, two lines (KS and K) being selected for short developmental rate and two lines (LS and L) for long developmental rate.

2) After 88 to 183 generations of selection, frequencies of the Aph-D-alleles D^1, D^2, and D^0 and of the Est-6-alleles 6^f and 6^s were estimated by isoenzyme studies.

3) With the exception of the reference line RE, the allele Est-6^s had been fixed. With the exception of the lines RE and LS, the allele Aph-D^2 had been eliminated.

4) In all lines the allele Aph-D^0 showed an almost constant frequency of about 0.197, only in line LS was the frequency much higher, at 0.265.

5) The selection had no clearly distinctive effect on the distribution of the Aph-D- and Est-6-alleles, since the same mode of selection resulted in different allele frequencies.

6) Selection restricts the degree of heterozygosity and the degree of allele polymorphism except for the Aph-D-alleles D^2 and D^0 in the line LS.

Introduction

The first publications of this series (Muhs 1975a,b) both dealt with isoenzyme frequencies in selection lines under different temperature conditions. The selection lines investigated in this and the following paper have five factors in common with the lines used for the temperature selection studies: (1) the founder stocks are the same four laboratory stocks; (2) the time of foundation from the same initial population differs only in a few generations; (3) they have the same non-selected reference line RE; (4) all the lines received the same food; (5) all of them were cared for by the same person. Therefore, the results of all investigations can be compared.

Since the present study deals with the distribution of the same alleles described in the first paper of this series (Muhs 1975a), it contributes additional facts for discussion of the different effects of selection and the different reactions of the population upon selection.

Materials and Methods

The general experimental technique, as well as the *Drosophila* stocks and the method of founding the initial population from which all selection lines were derived, have already been described (Muhs 1975a).

Selection lines: The factor used in selection for developmental rate was the time from oviposition to the emergence of the fly. Every generation had been founded with 240 female and male flies respectively. For a few days they were held in a population cage to allow random mating, then during 48 hours 24 food tubes were put on to the cage for oviposition. After oviposition, the flies were put aside and the food tubes brought on special boards to the incubator where the new generation developed.

Four of the 24 food tubes had been selected at random as control tubes. The emerged flies were counted daily in order to calculate the mean emergence day, the variance of the emergence period, and the total number of emerged flies. In each generation 480 newly emerged flies were selected from the other 20 food tubes.

Selection lines for short developmental rate KS and K: Six months after the line KS had been established, the line K was founded (Fig. 1). Both lines had the same mode of selection and had been selected up to the 183rd generation (line KS) or 156th generation (line K) (Table 1). Selection was mostly carried out during the first two days of emergence (8th and 9th day after oviposition). The first 480 flies founded the new population and were put into the cage for oviposition. The remaining flies were discarded. Fluctuations in the size of the population could not be avoided. These fluctuations could be due to seasonal influences (Tigerstedt 1969).

Selection lines for long developmental rate LS and L: The line LS is about half a year older than line L (Fig. 1). They were selected by the same mode of selection up to the 100th generation (line LS) or 88th generation (line L). In this case selection was made during the last 4 to 5 days of emergence (about the 14th to the 19th day after oviposition). The flies which emerged latest were used to found the new generation.

Isoenzyme and sample taking: The isoenzymes of the esterases and larval alkaline phosphatases and the sam-

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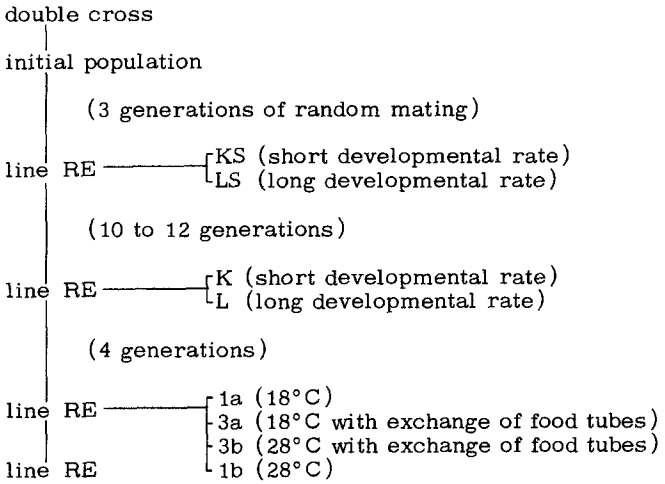

Fig. 1. Source of selection lines. Four laboratory stocks which were involved in the double cross founded the initial population (Muhs 1975a). The initial population was held at 25°C and designated RE. After the third generation of random mating in a population cage as first selection lines, the lines selected for short developmental rate (line KS) and long developmental rate (line LS) were derived from the line RE. About 10 to 12 generations later, again lines selected for short developmental rate (line K) and for long developmental rate (line L) were founded. After a further four generations the lines selected under different temperature conditions (lines 1a, 3a, 3b, 1b) were derived from the line RE. They have already been discussed (Muhs 1975a,b).

ple-taking have been described elsewhere (Muhs 1975a). It should be emphasized that the 480 flies selected for the new generation were used for a second oviposition, which was taken for the investigations reported here. The samples amounted to 256 larvae for the study on alkaline phosphatases or 256 flies for the study on esterases. Homogenizing and electrophoretic methods will be described elsewhere (Muhs 1975 c).

Results

The mode of inheritance, the nomenclature and the identification of the isoenzyme bands have already been described (Muhs 1975a). Two alleles of the Est-6-locus, 6^F and 6^S, had been found which can form the phenotypes 6F6F, 6F6S, and 6S6S; and three alleles of the Aph-D-locus, D^1, D^2 and D^0, which can form the phenotypes D1D1, D2D2, D0D0, D1D2, D1D0, and D2D0. As the alkaline phosphatases controlled by the Aph-D-locus probably had dimeric structure (Wallis and Fox 1968), the phenotypes can possess one, two or three isoenzyme bands, which are designated in the same way as the phenotypes.

Distribution of the alleles in the laboratory stocks and the reference line: Two of the four laboratory stocks contained both Est-6-alleles, while the other two stocks were homozygous for the allele Est-6^S. The allele D^1 of the Aph-D-locus was the most frequently observed in all stocks except in one where the allele D^2 prevailed. The allele D^0 was present in all stocks. Thus the Aph-D-locus was polymorphic in all stocks (see Muhs 1975a).

In the reference line the allele 6^S of the Est-6-locus was the most frequent one, the allele 6^F being found at a frequency of less than 0.01. For the Aph-D-locus, the allele D^1 was found most frequently. The allele D^2 occurred at a frequency lower than 0.01 and the allele D^0 at a frequency of 0.197. All alleles found in the laboratory stocks were present in the reference line, but the frequencies had changed considerably. Thus the alleles Est-6^F and Aph-D^2 had been observed only at rather small frequencies (see Muhs 1975a).

Selection lines: Table 1 gives the observed numbers of phenotypes, while Table 2 shows the corresponding estimates of the allele frequencies and their variances.

In all selection lines the allele Est-6^S had been fixed and the allele Est-6^F had been eliminated. Compared with the reference line which had maintained the allele 6^F at a low frequency, selection affected the fixation of the allele 6^S in all lines.

As in the reference line the allele Aph-D^1 occurred most frequently in all selection lines, which showed few differences in frequency except for the line LS. There, the frequency was the lowest (0.694), while in the other selection lines it varied from 0.803 to 0.875. The alle-

Table 1. Observed frequencies of Aph- and Est-phenotypes

Mode of selection	Selection line	Aph-phenotypes					Est-phenotypes		
		D1D1 D1D0	D1D2	D2D2	D0D2	D0D0	6F6F	6F6S	6S6S
25°C	Re	244	2	-	-	10	-	3	253
short	KS	246	-	-	-	10	-	-	256
short	K	252	-	-	-	4	-	-	256
long	LS	219	16	-	3	18	-	-	256
long	L	246	-	-	-	10	-	-	256

The electrophoretic method does not allow the phenotypes D1D1 and D1D0 to be distinguished, therefore both appear in one group.

Table 2. Estimates of the frequencies of the Aph-D- and Est-6-alleles and the variances

Mode of selection	Selection line	Frequencies of the Aph-D-alleles			Est-6-alleles		Variances of the estimates of Aph-D-alleles			Est-6-alleles
		D^1	D^2	D^0	6^F	6^S	D^1	D^2	D^0	6^F
25°C	RE	0.799	0.004	0.197	0.006	0.994	0.00094	0.00001	0.00093	0.00002
short	KS	0.803	-	0.197	-	1.000	0.00062	-	-	-
short	K	0.875	-	0.125	-	1.000	0.00043	-	-	-
long	LS	0.694	0.041	0.265	-	1.000	0.00060	0.00008	0.00063	-
long	L	0.803	-	0.197	-	1.000	0.00062	-	-	-

Table 3. Timing of Experiments

Mode of selection	Selection line	Time in generations (except for founder stocks) at the time of examination for isoenzyme frequencies			Overall selection time from the foundation of the selection line
		Est	LAP*	Aph	Years
25°C	RE**	132	148	162	6 1/4
short	KS	157	173	183	6 1/2
short	K	132	148	156	6
long	LS	86	95	100	6 1/2
long	L	77	84	88	5 3/4
Founder stocks		Dec.69	Feb.70	Feb.70	

* The results of the LAP-isoenzymes will be given in the following paper IV of this series.

** The numbers of generations for the reference line RE are roughly estimated, because RE was held permanently in a population cage.

le D^2 was eliminated in the selection lines KS, K, and L, but was observed in the line LS at a frequency of 0.041. This frequency is much higher than that of the reference line. Thus, 16 phenotypes D1D2 and 3 phenotypes D0D2 were found in line LS (Table 1).

The allele Aph-D^0 occurred in all selection lines and in the reference line at almost the same frequency of about 0.197. There were two exceptions: line LS had a frequency of 0.265, which was much higher than the average, while line K had the lowest frequency of 0.125.

It is striking that the lines which had been selected by the same method show differences. Lines LS and L are distinguished by the frequencies of the alleles D^0 and D^2, lines KS and K by a small difference in the frequency of the allele D^0. All selection lines differ from the reference line in lacking the alleles Est-6^F and Aph-D^2, except for the line LS which contains the allele D^2.

Timing of experiments: Selection lines had been selected during long periods of up to 6 1/2 years. For technical reasons not all experiments were done at the same time, so that the single lines were examined at different times. Usually the esterases were studied about one year prior to the alkaline phosphatases. For the "long" lines L and LS this difference amounts to 11 to 14 generations, for the "short" lines K and KS it amounts to 24 to 26, and even 30 generations for the reference line RE (see Table 3). However, the studies on esterases as well as alkaline phosphatases have been carried out successively within half a year, so we can assume that the allele frequencies had not changed much and the results can be compared.

The founder stocks were analysed in 1969 and 1970, about 5 to 6 years after they had been used to found the initial population. During this time there may have been a shift of allele frequencies, but we assume that the founder stocks had maintained the balanced allele polymorphisms (see Muhs 1975a).

Discussion

Characters of selection: As an artificial character of selection we used the timespan of development, i.e. the interval from oviposition to the emergence of the young imago. In contrast to the lines selected under different temperature conditions, here the only individuals able to reproduce are those with an extremely short (at the lines KS and K) or an extremely long (at the lines LS

and L) developmental rate. For each generation (apart from some exceptions where only 240 individuals were used) 480 flies had been selected from populations of about 6000 individuals, corresponding to a percentage of about 8% (Tigerstedt 1969).

The average life time of a fly can be more than two months. Our selection, however, was made at once after emergence (normally the 8th to 10th day for lines KS and K and between the 14th and 19th day for the lines LS and L), so viability was only tested in the first third of the whole life span. The further fate of the individual in the population after reaching maturity remained unknown.

Two essential stages can be distinguished in the development from egg to emergence of the flies; namely the larval and the pupal stages. The development of the larvae takes place under intense competition for food and living space because of the observed high density of larvae in the food tubes, so we may suppose that the competitive ability of the larvae plays an importent role (Mather and Cooke 1962; Parsons 1958). However, contrary to the position in the temperature selected lines (which are permanent populations), every larva has a chance to get fresh food not used by the preceding generations because each generation is newly founded and receives enough food. The pupal stage lasts from 3 days to about 7 to 10 days (e.g. in lines selected for long developmental rate). In this stage pupae are independent of food. They run through a canalized development or homeorhesis (Waddington 1957). In the lines selected for developmental rate all the pupae usually survive and the young flies have a chance to emerge, but in the temperature selected lines only those pupae survive which have not submerged in the food constantly turned over by the larvae.

The characters of selection are closely correlated with fitness with the following peculiarities: the chance to survive until maturity and fertility (Knight and Robertson 1957). Fast development is advantageous for the larvae but the consequence of excessive shortening of the developmental rate is decreasing fertility of the flies (Hiraizumi 1961). Selection characters closely correlated with fitness only show small tolerance (Marien 1958; Clarke et al. 1961) and have small additive genetic variance (Falconer 1960). Thus selection for short developmental rate will soon reach a limit (Robertson 1960), where further selection is accompanied by decreasing fitness (James 1962). Fitness increases best by the organization of new adaptive gene complexes. In these lines fitness had increased, except in line L (Tigerstedt 1969), so we can assume that new gene complexes had been organized.

Reaction upon selection: In this study selection for a long or short term of development, as well as selection under different temperature conditions (Muhs 1975a), caused the elimination of the Est-6^F-allele.

Selection for short developmental rate eliminated the Aph-D^2-allele, too, but for long developmental rate only in line L. On the other hand the line LS possessed the highest frequency of the allele D^2 of all lines, including those selected under different temperature conditions. This differing reaction to the same mode of selection can be explained by the fact that the line LS was derived shortly after the foundation of the initial population, while line L was obtained about 10 to 12 generations later (see Fig.1). At that time the degree of heterozygosity of the reference line RE could already have been restricted so that the frequency of the allele D^2 decreased to the value 0.01, even less in the line RE. Elimination in the following generations may be explained by drift even if the allele D^2 should have an advantage of selection for long developmental rate. Line LS reacted upon selection only to a small degree. Possibly the line had been able to resist the pressure of selection without losing fitness. On the other hand, line L had reacted upon selection with loss of additive genetic variance and of fitness (Tigerstedt 1969). There seems to be a correlation between allele polymorphism and fitness.

It can not be explained conclusively why line LS, which shows a higher fitness value than line L, had enriched the silent allele D^0. Obviously a population selected for a character which is contrary to natural selection has to accept a higher degree of genetic load as long as it can resist the selection pressure and maintain fitness.

In the lines KS and K selected for short developmental rate there were no significant differences observed in the distribution of the Aph-D-alleles. Why these lines also had maintained the silent allele D^0 can not be explained. The allele D^0 had also been found in the temperature selected lines (Muhs, 1975 a) and it was discussed whether there was a different mechanism maintaining the silent allele.

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Dr. Hans-J. Muhs
Forstbotanisches Institut der
Albert-Ludwigs-Universität
Bertoldstr. 17
D-78 Freiburg (Germany/BRD)