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Isoenzyme Frequencies in Long-term Selection Lines of *Drosophila melanogaster* II. Isoenzyme Frequencies of Leucine Aminopeptidases (LAP) in Selection Lines under Different Temperature Conditions*

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Summary. 1) Permanent population lines of *Drosophila melanogaster*, derived from a double cross of 4 laboratory stocks, were selected under different temperature conditions (18 °C and 28 °C) for at least 125 generations.

2) The isoenzymes of the leucine aminopeptidases (LAP) controlled by two closely linked loci on the third chromosome were investigated: LAP-A-locus in position 98.0 and LAP-D-locus in position 98.3. Thus the frequency of linkage groups could be estimated by the isoenzyme patterns.

3) In the laboratory stocks the linkage groups A^0D^f, A^fD^f, A^sD^s, A^0D^s and A^sD^f were present. For the selection lines and reference line the last two linkage groups were absent.

4) The reference line had maintained an allele polymorphism with the frequencies of the linkage groups: $p = 0.145$ for A^0D^f, $q = 0.605$ for A^fD^f, and $r = 0.250$ for A^sD^s. The reference line had lost the linkage groups A^0D^s and A^sD^f. This fact can be explained by a change of the gene pool in the first generations after the double cross.

5) The selection lines differed from the reference line by higher frequencies of the linkage group A^sD^s.

6) Among the selection lines the frequencies were not very different. Selection showed only small effects. All selection lines had reacted to selection in the same way: the linkage group A^fD^f increased and the linkage group A^sD^s decreased.

7) A high frequency of the silent allele A^0 was observed in all lines. The maintenance of this allele can not be explained as yet.

Introduction

The isoenzyme of the leucine aminopeptidases (LAP) which were investigated here are controlled by two closely linked loci on the third chromosome: the LAP-A-locus in position 3-98.0 and the LAP-D-locus in position 3-98.3 (Falke and MacIntyre, 1965; O'Brien and MacIntyre, 1971). These loci can be regarded as markers of the third chromosome. Effects of recombination and selection which are restricted to this small section of the third chromosome can be observed.

The selection lines had been derived from a double cross of four laboratory stocks and were selected for temperature adaptation. All lines were kept at constant temperature in incubators, two of them at 18°C, another two at 28°C, and the reference line at 25°C. One of the lines at 18°C and one of the lines at 28°C were specially treated so as to maintain a constant, artificial migration of genes. The other lines, at 18°C and 28°C did not get such treatment. Changes of population structures can be observed at two steps of selection by comparing

(1) the laboratory stocks with the reference line and

(2) the reference line with the selection lines.

The investigation described in the first publication of this series (Muhs, 1975a) dealt with the distribution of the isoenzymes controlled by the Est-6-locus (Wright, 1963) and the Aph-D-locus (Wallis and Fox, 1968) in the same selection lines. Both loci lie on the third chromosome, too. A comparison of the results with those obtained here is of special interest, because both isoenzyme groups have a different physiological meaning.

Materials and Methods

The general experimental technique, the *Drosophila* stocks and the selection lines under different temperature conditions have already been described (Muhs, 1975a). About 15 and 25 generations following the investigation of esterases and alkaline phosphatases, the selection lines were analysed for the leucine aminopeptidases. The random samples (256 pupae) were collected in the same way as described in the first publication of this series (Muhs, 1975a). For the electrophoretic investigation, pupae which had reached the last stage of pupation were used. At this stage the leucine aminopeptidases show their highest activity (Sakai et al., 1969).

* This study is a part of a thesis (Faculty of Forestry, University of Freiburg (Germany)).

Isoenzymes: The isoenzymes are controlled by two closely linked loci. In both loci several different alleles are present: for the LAP-A-locus the alleles A^F (controlling a fast migrating isoenzyme), A^S (controlling a slow migrating isoenzyme), and A^O (homozygotes show no detectable enzyme activity in the region of the A-bands, heterozygotes have reduced staining intensity compared with the homozygotes A^FA^F or A^SA^S); for the LAP-D-locus the alleles D^F (controlling a fast migrating isoenzyme), D^S (controlling a slow migrating isoenzyme), D^X (controlling a very slow migrating isoenzyme), and D^O (homozygotes show no detectable enzyme activity in the region of the D-bands, heterozygotes have only half the staining intensity of that of homozygotes D^FD^F, D^SD^S, and D^XD^X) (Muhs, 1974b; 1975b). In the selection lines the alleles D^X and D^O occurred only very rarely and they are not considered here. Since both loci are closely linked and the isoenzymes controlled by them can be identified in the same zymogram, the linkage group for each individual could be determined. In all selection lines the following linkage groups were mostly observed: A^OD^F, A^FD^F and A^SD^S. These three linkage groups can form six genotypes (table 2). The genotypes A^OD^F/A^FD^F and A^FD^F/A^FD^F, as well as the genotypes A^OD^F/A^SD^S and A^FD^F/A^SD^S, could not be clearly distinguished in our zymograms. Therefore we had to arrange the phenotypes in four groups, in which the groups II and IV include two phenotypes each:

Group I : AOAO/DFDF
Group II : AFAF/DFDF and AOAF/DFDF
Group III : ASAS/DSDS
Group IV : AOAS/DFDS and AFAS/DFDS

Homogenizing and electrophoretical methods have been described elsewhere (Muhs, 1975b). The staining method was slightly modified after Beckman and Johnson (1964). A method which allows a reliable and rapid identification of the isoenzyme bands AF and AS has been described by Muhs (1974a).

Results

the investigation isoenzymes were found which are ntrolled by the linkage groups A^OD^F, A^FD^F, A^SD^S, $^OD^S$, A^SD^F, and A^SD^X. The linkage group A^SD^X was ly observed twice in the reference line RE and could disregarded.

The first three linkage groups were found in the laratory stocks which had been used to found the initial pulation of the selection lines as well as in the selecon lines. The linkage groups A^OD^S and A^SD^F were on- observed in the laboratory stocks. Linkage groups n only be detected if the individual is homozygous. us it is possible that there are some individuals with e linkage group A^OD^S and A^SD^F in the phenotype oup IV in which the double heterozygotes are enclosed.

Individuals belonging to this phenotype group were und in the selection lines but no individuals which ere homozygous for the linkage group A^OD^S or A^SD^F. may be concluded that these two linkage groups do not ist in the selection lines or they exist only at a low equency, so they were not taken into account when calulating the gene frequencies.

Laboratory stocks: Before and after foundation of the initial population from which all selection lines were derived the laboratory stocks have been kept under constant conditions in the laboratory. So it may be assumed that the observed allele polymorphisms are balanced.

The distribution of the linkage groups in the four laboratory stocks is shown in Table 1. Except in the stock 10 A Käs which had fixed the linkage group A^OD^F, all stocks were polymorphic for two or three linkage groups. The most frequent is the linkage group A^OD^F in the stocks 10 A Käs and Käs 60. In the line 7 AT this linkage group is present, but the linkage group A^SD^S prevails. The stock 9 DE lacks the linkage group A^OD^F,

Table 1. The Distribution of the Linkage Groups in the Four Laboratory Stocks of *Drosophila melanogaster*

Stock	A^OD^F	A^FD^F	A^SD^S	A^OD^S	A^FD^S	A^SD^F
7 At	+	-	++	-	-	+
Käs 60	++	+	-	+	-	-
10 A Käs	+++	-	-	-	-	-
9 DE	-	-	++	+	-	-

A plus sign means that the respective linkage group was found in the stock. Two or three plus signs point to linkage groups which were most frequent. A minus sign shows that the respective linkage group was not found or identified. Since only 64 pupae from each stock were examined, it cannot be excluded that those linkage groups marked by minus are nevertheless present in low frequencies. The first three linkage groups in table 1 were found once more in the selection lines, the other three were not.

Table 2. Relation between Genotype, Phenotype and Expected Frequency

Genotype	Phenotype	Phenotype Group	Expected Frequency
A^OD^F/A^OD^F	AOAO/DFDF	I	p^2
A^OD^F/A^FD^F	AOAF/DFDF	II	2pq
A^FD^F/A^FD^F	AFAF/DFDF	II	q^2
A^SD^S/A^SD^S	ASAS/DSDS	III	r^2
A^OD^F/A^SD^S	AOAS/DFDS	IV	2pr
A^FD^F/A^SD^S	AFAS/DFDS	IV	2qr

the linkage group A^SD^S being most frequently observed in this stock. The linkage group A^FD^F could only be found in the stock Käs 60. Besides these linkage groups which occurred in the selection lines also, two linkage groups exist only in some stocks: the linkage group

Table 3: Table 3 shows the observed and expected numbers of the phenotype groups. The χ^2_1-test is a goodness of fit test, the table value is 3.84 for one degree of freedom (df) and 95% probability. The χ^2_2-test is a heterogeneity test against the reference line RE, the table value is 7.81 for 3 df and 95% probability

Mode of Selection (°C)	Selection Line	Phenotype Groups: Observed Numbers I	II	III	IV	Expected Numbers I	II	III	IV	χ^2_1	df	χ^2_2	df
25	RE	5	132	14	103	5.33	137.54	15.88	95.25	1.097	1		
18	1a	5	193	3	54	5.00	193.37	3.54	53.09	0.169	1	32.860	3
18	3a	11	179	6	60	10.85	177.18	5.22	62.75	0.258	1	25.896	3
28	3b	6	181	6	63	5.99	179.84	5.61	64.56	0.412	1	22.600	3
28	1b	12	188	1	55	11.83	188.67	3.38	52.12	1.840	1	40.531	3

Table 4. Estimates of Gene Frequencies and their Variances

Mode of Selection	Selection Line	Frequencies: Linkage Group A^OD^F	A^FD^F	A^SD^S	Variances: Linkage Group A^OD^F	A^FD^F	A^SD^S
25 °C	RE	0.145	0.605	0.250	0.00056	0.00171	0.00097
18 °C	1a	0.140	0.742	0.118	0.00110	0.00096	0.00020
18 °C	3a	0.206	0.651	0.143	0.00105	0.00093	0.00024
28 °C	3b	0.153	0.699	0.148	0.00111	0.00095	0.00029
28 °C	1b	0.215	0.670	0.115	0.00245	0.00218	0.00016

A^OD^S in the stocks Käs 60 and 9 DE and the linkage group A^SD^F in the stock 7 AT. The linkage group A^FD^S was not found in these stocks.

Selection lines: The six different genotypes are shown in Table 2 together with the corresponding phenotypes, the group of phenotypes and the expected frequencies. Here the frequency of the linkage group A^OD^F is marked by p, that of A^FD^F by q, and that of A^SD^S by r.

Table 3 shows the observed and expected frequencies for each of the four phenotype groups, and Table 4 the corresponding frequencies of the linkage groups. In line RE, which can be regarded as reference line for all selection lines, all phenotypes were found. More than half of the observed numbers (132 out of 256) were of phenotype group II, while there were 103 individuals of phenotype group IV. The phenotypes I and III have been found only 5 and 14 times, respectively. From these data the linkage group frequencies were computed by the method of maximum likelihood. The corresponding frequencies were: p = 0.145 for A^OD^F, q = 0.605 for A^FD^F, and r = 0.250 for A^SD^S.

It is common to the selection lines that they differ mainly in the frequencies of the phenotype groups II and IV compared with the reference line. In all selection lines the phenotype group II appeared most frequently, with more than two-thirds of the total numbers. The observed numbers varied between 179 (line 3a) and 193 (line 1a). In phenotype group IV the selection lines showed frequencies between 54 (line 1a) and 63 (line 3b). In the phenotype groups I and III the observed numbers varied from 5 to 12 and from 1 to 6, respectively. Here the frequency of phenotype group I of line RE was equal to the lowest of the selection lines, and the frequency of the phenotype group III of the line RE was much higher than those of the selection lines.

Between the selection lines only small differences could be found. The most frequent linkage group A^FD^F varied from 0.651 (line 3a) to 0.742 (line 1a). Here the "cold" line (1a) showed the highest frequency and the "warm" line (1b) a rather low one. The linkage group A^OD^F was less frequent in line 1a ("cold") and showed higher frequency in the "warm" line (1b). The lines with exchange of food tubes (3a and 3b) had rather similar frequencies. They showed a somewhat

higher frequency of linkage group A^SD^S than the selection lines without exchange of food tubes. Concerning the linkage group A^OD^F the lines 3a and 3b had frequencies lying between those of the lines 1a and 1b.

The goodness of fit of the observed numbers to the values expected from the Hardy-Weinberg rule is good, and is given by χ^2_1-values in Table 3. The difference of the selection lines from the reference line was tested with the χ^2-tests. The values, which are shown by the χ^2_2-values in Table 3, are very high and significant, and clearly distinguished all the selection lines from the reference line RE.

When testing for homogeneity in the selection lines 1a, 1b, 3a and 3b, a χ^2-value of 10.54 was calculated, which, with 9 degrees of freedom, was smaller than the scale value of 16.90, i.e. the lines selected for different temperatures are homogeneous.

In order to test for the possible influence of the taking of random samples on the results, an analysis of variance with three-way-classification was carried out. The transformation $\sqrt{1/2 + x}$ was generally used, in order not to have to compute with unfilled classes. The sources of variability can be split up in the following way: graduated oviposition (A) (each population was allowed to lay eggs for two days, 4 times in succession), food tubes (B), day of sampling (C), the interaction between graduated ovipositions and food tubes (AB), the interaction between graduated ovipositions and day of sampling (AC), the interaction between food tubes and day of sampling (BC). At the 5% level, only the F-values of the phenotype group I of the line RE for the interactions (AB) and (BC) are significant. All other F-values including those of the main effects are not significant.

Thus it can be assumed that an influence of the sources of variability does not exist, and that the random samples were taken without bias. The F-values were calculated by means of the approximate F-test according to Satterthwaite (from Ostle, 1964).

Discussion

Characters of selection and isoenzymes: The characters of selection and their relation to natural selection were described in the first publication of this series (Muhs, 1975a). The importance of the leucine aminopeptidases, which have other functions during development than those of the esterases and alkaline phosphatases, will be discussed in more detail. The leucine aminopeptidases are particularly active during the massive histolysis in the pupal stage. They are detectable in larvae of the third instar and in young adults (Sakai et al., 1969), but the staining intensity of the isoenzyme bands is considerably smaller than in the pupal stage. It can be concluded that the leucine aminopeptidases play an essential part at the pupal stage of metamorphosis.

Waddington (1957) described the part of the ontogeny, which is independent of food and other external conditions, as a canalized development or homeorhesis, which automatically seems to follow a pre-designed path. Bakker (1959, quoted from Tigerstedt, 1969) found that variation of the environmental conditions which exist before this stage indeed influences the size and form of the adults, but not the length of the canalized development. My own unpublished results show that inbred lines which were homozygous for the allele D^S of the LAP-D-locus had a longer period of development than those which were homozygous for the allele D^F. The delay in development was achieved by prolonging the pupal stage up to half a day. This indicates that genetic constitution determines the length of the canalized development. However, a short period of pupation may be advantageous for the emerging flies. This may be an explanation for the fact that the allele D^F occurs much more frequently than D^S.

Laboratory stocks: In 1963 the laboratory stocks were used to found the initial population from which all selection lines were derived. The isoenzyme investigation was carried out at the end of 1970. In the meantime the laboratory stocks have been kept under constant conditions in the laboratory, so that a frequency shift, as well as the loss or the manifestation of a linkage group, is conceivable. But since the laboratory stocks, during the course of many years, had adapted to the laboratory environment, it is to be expected that a balance between the linkage groups in each single line has arisen which to a great extent has remained stable. This may be true for all stocks except the stock 10 A Käs, which had fixed the linkage group A^OD^F.

Therefore we may assume that at the time when the initial population was founded, and at the time of isoenzyme investigation, the allele frequencies were nearly the same. If this is true, the gene pool of the initial population, which was composed of a quarter of each gene pool by crossing the four laboratory stocks, had changed drastically compared with the gene pool of the reference line RE.

During the time of selection both the linkage groups, $A^{O}D^{S}$ and $A^{S}D^{F}$, were lost. The linkage group $A^{F}D^{F}$, which occurred only in the stock Käs 60, had reached the highest frequency in all selection lines. Both linkage groups $A^{O}D^{F}$ and $A^{S}D^{S}$, which were the most frequent in the stocks, had been reduced to a frequency of less than 0.250. This drastic change in the gene pool of the initial population can be explained by the large fluctuations of population size, which showed an alternating sequence of overcrowding and breakdown (Muhs, 1975a). This interpretation can also explain why all selection lines show similar allele frequencies. The lines selected under different temperature conditions had been obtained about 17 to 19 generations after founding the initial population. During these first generations the striking changes in the gene pool may have happened.

Selection lines: If one compares the results of Levins (1965), who studied the adaptive possibilities of three species of Drosophila, with out data showing slight differences in the frequencies of the linkage groups, it can partly be explained by tolerance in face of a broad niche and a high individual homeostasis, since all four selection lines were able to keep the same allele polymorphism on the LAP-A- and LAP-D-locus. The χ^2-tests (see table 3), however, point to a clear difference of the line RE from all others, so that here a change in the genotype frequencies must be assumed. In contrast, the selection lines are similar in their frequencies and their homogeneity as shown by the χ^2-test. These facts can only be interpreted by the assumption that the adaptation to temperature could not be achieved by mere tolerance and individual homeostasis, but that the reaction to different temperatures took place in the same way in the cases of the LAP-A- and LAP-D-locus.

The ability to react to selection pressures which suddenly arise is called pre-adaptation and is an essential characteristic of a population. This ability could be present also in the lines selected here, although evidence by exact measurement of, for example, the fitness value could not be given at the different temperatures. Genetic variability is a prerequisite for pre-adaptation. On the LAP-A- and LAP-D-locus allele polymorphism was indeed observed. This indicates that mechanisms maintaining allele polymorphism are present. This will be generally discussed in paper IV of this series (Muhs, 1975c).

The silent allele A^{O}: A remarkable result is that the allele A^{O} has, for a silent allele, a high frequency in all lines as well as in three of the four laboratory stocks. In the stock 10 A Käs this allele alone was found (see table 1), so that it can be assumed that the carriers of this silent allele have a great selective advantage either in themselves or in particular linkages (here the linkage group $A^{O}D^{F}$). At present the fitness-values of the linkage group have not been estimated, so that further interpretation is not possible.

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