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Isoenzyme Frequencies in Long-term Selection Lines of *Drosophila melanogaster* I. Isoenzyme Frequencies of the Esterases (Est) and Larval Alkaline Phosphatases (Aph) in Temperature Selected Lines*

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Summary. 1) Lines of *Drosophila melanogaster* derived from a double cross of four laboratory stocks and selected for more than 125 generations were investigated by the isoenzyme method. The reference line RE was held constantly at 25 °C, the selection lines 1a and 3a had a constant environment at 18 °C, the selection lines 1b and 3b at 28 °C. Additionally a permanent artificial migration was maintained between the selection lines 3a and 3b by exchange of food tubes.

2) The isoenzymes of the esterase controlled by the Est-6-locus and the larval alkaline phosphatase controlled by the Aph-D-locus were analysed.

3) Two of the laboratory stocks contain both alleles 6^f and 6^s of the Est-6-locus, the other two are homozygous for the allele 6^s. All three alleles D^1, D^2, and D^0 of the Aph-D-locus are present in each of the four laboratory stocks, except that stock 7 At lacks the allele D^2.

4) In the reference line RE both Est-6-alleles and all three Aph-D-alleles are present, but the alleles 6^f and D^2 occur at very low frequencies. The alleles 6^s and D^1 are the most frequent alleles, the silent allele D^0 occurs at a frequency of 0.197.

5) In the selection lines the alleles 6^s and D^1 are the most frequent alleles, too. The allele 6^f is lacking in all selection lines. In the lines 1a and 3b the allele D^2 is missing, but this allele was found in the lines 1b and 3a at very low frequency. The silent allele D^0 is common to all selection lines, with the nearly constant values of 0.141 to 0.177. These frequencies are smaller than in the reference line.

6) All selection lines have rather similar allele frequencies and can be compared with those of the line RE (except the allele 6^f). But these allele frequencies differ from those of the four laboratory stocks. This is attributed to a change in the linkage groups by recombination and the organisation of new adaptive gene complexes during the first generations after the foundation of the double cross.

7) Selection for differing adaptation to temperature has only a small effect on the observed loci.

8) The relatively high frequency of the silent allele D^0 found in all lines is discussed with regard to selective neutrality, partial selectivity, and a mutation-selection balance.

Introduction

The reaction of a population on selection will be determined by several factors, such as population structure, the mode of adaptation of the population, and the type and intensity of selection. The latter can be regulated by the experimenter. Although in some cases it has been shown that *Drosophila melanogaster* has good tolerance within a broad niche, as well as high individual homeostasis and a low degree of polymorphism (Levins, 1965), the mode of adaptation remains mostly unknown.

The structure of the gene pool is another unknown factor but there are methods available, especially the investigation of isoenzymes, by which exact data could be obtained to characterize the population structure in some particular loci or gene complexes. Such data give an idea of the composition of the gene pool. By this method the population structures of artificial, and even natural, populations of several *Drosophila* species have been characterized (Hubby and Lewontin, 1966; Johnson et al., 1966; Lewontin and Hubby, 1966; Stone et al., 1968; Ayala et al., 1972; Brncic et al., 1969; Prakash et al., 1969; Berger, 1971; Kojima et al., 1972).

In this paper long-term selection lines of *Drosophila melanogaster* have been studied in order to characterize the structure of four polymorphic loci which give some information on the changes in allele frequencies caused by selection. The changes of allele frequency were obtained by analysing the lines for each group of isoenzymes. The following isoenzyme groups were chosen: the nonspecific esterases controlled by the Est-6-locus, the larval alkaline phosphatases controlled by the Aph-D-locus and the leucine aminopeptidases controlled by the LAP-A- and LAP-D-locus. The founder stocks were polymorphic for one or more of these loci.

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The results will be given in a series of four papers: papers I and III will deal with the esterases and larval alkaline phosphatases, papers II and IV with the leucine aminopeptidases.

As the allele frequencies were analysed only once during the life of each selection line, no data on genetic changes during the whole period of selection could be obtained. Comparison with the reference line was possible, however, since it was examined at the same time as the selection lines. There were no exact data on the allele frequencies of the initial population (the reference line just after it was founded by a double cross of four laboratory stocks), from which all selection lines were derived, but such data could be reconstructed to a certain extent by analysing the four laboratory stocks from which the initial population was derived.

The selection lines can be divided into two groups: (1) the temperature selected lines and (2) the lines selected for developmental rate. The temperature selected lines had large population sizes and overlapping generations, i.e. backcrosses were possible at any time. The lines selected for developmental rate consisted of medium-sized populations with non-overlapping generations. The results of the first group of selection lines will be given in papers I and II, and of the second group in papers III and IV. Tigerstedt (1969) has studied the lines of the second group in earlier generations. His results are of particular interest for the interpretation of the data presented in these papers.

A general discussion of all selection lines will be given in paper IV. In particular, the mechanisms maintaining allelic polymorphism are discussed with regard to the allele distribution.

Materials and Methods

General experimental technique: All populations were kept in population cages of plexiglas (acrylic plastic) 20 × 20 × 10cm in size. The cage had 24 sockets for food tubes 2.5cm in diameter and 8.5cm in length. Each tube contained about 10g food prepared from maize (semolina), syrup, alcohol, dead bakers' yeast, agar, and nipagin. A special apparatus was used for supplying the tubes with equal amounts of food (see Tigerstedt, 1969). The food was stored in a refrigerator for not longer than six weeks. Before use the food was warmed to room temperature and supplied with a piece of pulp (laboratory quality) and with 3 to 4 drops of living yeast suspension provided with some sugar.

Selection was carried out under ether narcosis. The flies were then allowed to recover for two days to overcome sterility and inhibition of egg-laying as a consequence of ether shock. All populations were kept in incubators and were removed only for selecting and feeding.

Drosophila stocks: In 1963 the project was started by K. Stern in the Institute of Forest Genectics at the University of Stockholm. Four laboratory strains of *Drosophila melanogaster*[1] were used as founder stocks to establish a double cross and its reciprocals according to the following scheme:

(7 AT × Käs 60) × (10 A Käs × 9 DE).

Each cross consisted of equal numbers of 250 female and 250 male flies. This means that some 500 flies from each of the four founder stocks were used in the double cross. All crosses were carried out at 25 °C in population cages, in which mating was assumed to occur at random.

The initial population, which resulted from the double cross, was later called RE and maintained in a population cage at 25 °C.

The different investigated selection lines were derived from the line RE. After 3 generations of random mating the lines selected for developmental rate were established. These lines will be described in paper III. About 17 to 19 generations after the double cross had been carried out the temperature selected lines were founded. Before and during establishment of the selection lines the line RE showed fluctuations in population size, which disappeared after some 10 to 15 generations.

In 1964 the whole material was transferred to the Institute of Forest Genetics at Schmalenbeck. From 1968 to 1971 the investigations were done by the isoenzyme-technique.

In addition to the selection lines, the four founder stocks were also analysed. These stocks were kept under constant laboratory conditions before and after the foundation of selection lines. In 1969 the stocks were obtained from the Institute of Genetics at the Universtiy of Stockholm for isoenzyme investigation.

Temperature selected lines: Four temperature selected lines were simultaneously derived from the line RE. Two of them (the lines 1a and 3a) were kept at 18 °C ("low-temperature"), the other two lines (1b and 3b) at 28 °C ("warm temperature"). All lines were held in population cages. Twice a week they were fed with 4 food tubes. After 21 days the oldest food tubes were renewed, it being assumed that during this time even individuals which developed extremely slowly had had a chance to reproduce.

At every feeding, food tubes of the lines 3a and 3b were exchanged in such a way that two of the four tubes, which had been put on the cage the preceding week, were brought from the cage of line 3a to that of line 3b, and vice versa. In the exchanged food tubes all stages of development of the fly, from egg to imago, could be found. This exchange was made supposing that all four food tubes of the same feeding had equal numbers of eggs, larvae, and pupae. Altogether, the food tubes of the lines 3a and 3b have been exchanged 542 times.

Taking of samples: For the isoenzyme investigations four graduated ovipositions of the populations were prepared. Each oviposition comprised 4 food tubes, into which the populations were allowed to lay eggs for two days. The development of the larvae and flies took place in incubators at 25 °C. On 4 different days, samples of 4 larvae or 4 flies were taken out of each tube. The total number of am-samples consisted of 256 larvae or 256 flies (4 ovipositions × 4 tubes × 4 days × 4 larvae or flies).

For the investigation on the alkaline phosphatases larvae of the third instar, and for that of the esterases, 1 to 4 day-old flies were used.

Electrophoretic method: The method of homogenizing, the electrophoresis on starch gel, and the staining method are described elsewhere (Muhs, 1975).

Isoenzymes: The investigations were made to find out which isoenzymes existed in an individual and how many individuals with the same isoenzymes could be found in one line. Only the isoenzymes 6F and 6S were examined,

[1] Courtesy of Dr. Ramel, Institute of Genetics at the University of Stockholm.

which are controlled by two alleles of the Est-6-locus (Wright, 1963). Concerning the isoenzymes of the larval alkaline phosphatases, the isoenzymes D1D1, D2D2, D1D2, D1D0, D2D0, and D0D0 were analysed, which are controlled by three alleles of the Aph-D-locus. Here, D0D0 had no detectable activity in the zymogram.

The Est-6-locus has been mapped on the third chromosome in position 36.8 by Wright (1963) and the Aph-D-locus on the third chromosome in position 47.3 by Wallis and Fox (1968).

Results

Alleles and isoenzymes: Both alleles 6^F and 6^S of the Est-6-locus coding for esterase isoenzymes are able to form three genotypes: 6^F6^F, 6^F6^S, and 6^S6^S. The corresponding phenotypes are called 6F6F, 6F6S, and 6S6S. The phenotypes are easily recognized because the phenotypes 6F6F and 6S6S show one isoenzyme band each but with a difference in migration rate. The phenotype 6F6S shows both bands.

In the zymogram, up to 7 esterase isoenzyme bands have been observed. The isoenzymes 6F and 6S appeared as red bands because of their specificity to 2-naphthylacetate. On staining the gel with a mixture of 1- and 2-naphthylacetate, most of the other esterase isoenzymes were coloured black because of their specificity to 1-naphthylacetate. So did the esterase isoenzymes, controlled by the Est-C-locus (Beckman and Johnson 1964b), which were situated next to the esterase bands 6F and 6S in the zymogram. This staining technique allowed the bands controlled by the Est-6-locus to be easily identified. In addition, all selection lines showed only one isoenzyme band controlled by the Est-C-locus, so this band could be used as a marker for rapid recognition of the fast moving band 6F with respect to the slow moving band 6S during the serial investigations.

The Aph-D-locus coding for alkaline phosphatases has three alleles which are marked D^1, D^2, and D^0. The allele D^1 controls the fast migrating isoenzyme band, D^2 the slow migrating one, and D^0 is a silent allele. Because of the dimeric structure of the isoenzymes controlled by the Aph-D-locus four different forms have been found and named D1D1, D1D2, D2D2, and D2D0. D1D2 and D2D0 are called hybrid enzymes. D0D0 is an inactive protein which obviously has the position of D1D1 in the zymogram. Heterozygotes D^1D^0, which possess both the band of D1D1 and the hybrid enzyme of D1D0, only show one band in the position of D1D1. Therefore the heterozygote D^1D^0 cannot be distinguished electrophoretically from the homozygote D^1D^1. However, since D2D0 appears only together with D2D2, this hybrid enzyme can easily be recognized. Heterozygotes D^1D^2 possess three isoenzymes D1D1, D1D2, and D2D2. The hybrid enzyme band of D1D2 appears between the isoenzyme bands of D1D1 and D2D2, but closer to the D1D1 band instead of mid-way between D1D1 and D2D2. The hybrid enzymes D1D2 and D2D0 have the same position in the zymogram. They are easily distinguished because D1D2 is accompanied by both bands D1D1 and D2D2, while D2D0 is only accompanied by the band D2D2.

Distribution of the alleles in the founder stocks: In order to get an idea of the distribution of the alleles in the laboratory stocks which were used for the foundation of the initial population, the laboratory stocks were examined. Before and after the foundation of the selection lines the laboratory stocks have been kept under the same conditions. It is conceivable that during this time the allele frequencies could have shifted or even fixation of an allele could have taken place. The results, however, point towards balanced allele polymorphisms (see Table 1). In Table 1 no frequencies are given because only 64 individuals of each stock were tested, not sufficient for an estimate. Therefore, a minus sign in the table does not mean that this allele does not exist; it only means that it was not found or recognized.

The allele D^1 of the Aph-D-locus is the most frequent in all laboratory stocks except in the stock 10 A Käs. The allele D^2 is not present in the stock 7 AT, but in the stock 10 A Käs is the most frequent one. The silent allele D^0 occurs in all stocks with almost the same frequency.

The allele 6^S of the Est-6-locus is the most frequent one in all stocks. The allele 6^F can be found in the stocks 7 At and Käs 60 but is absent from stocks 10 A Käs and 9 DE. Both alleles 6^F and D^2, which have become rare in the selection lines, are well represented in the stocks. Each stock is polymorphic for one of the loci examined here, some are even polymorphic for both loci.

Table 1. Distribution of the Aph-D- and Est-6-alleles in the *Drosophila*-stocks, which are the founder stocks for the selected lines

Stock	Alleles of the Aph-D-locus			Alleles of the Est-6-Locus	
	D^1	D^2	D^0	6^F	6^S
7 AT	++	-	+	+	+
Käs 60	++	+	+	+	+
10 A Käs	+	+-	+	-	++
9 DE	++	+	+	-	++

++ the allele is observed with a frequency more than 0.5;
+ the allele is observed with a frequency less than 0.5;
- the allele is not observed.

Frequencies of isoenzymes and alleles in the selection lines: Table 2 lists the observed frequencies of the phenotypes for the isoenzymes of the esterases and larval alkaline phosphatases. Concerning the esterases, in all lines except the reference line RE only the isoenzyme 6S has been found. In the line RE, only three of 256 examined individuals have been found to have both isoenzymes 6S and 6F, so the allele 6^F shows a frequency of less than 0.01 (see Table 3). This means that the allele 6^S has been fixed in all selection lines.

The band D1D1 of the larval alkaline phosphatases is the most frequently observed (see Table 2). In addition, 2 or 3 of the phenotypes D1D2 have been found in the lines RE, 3a, and 1b. The frequency of the allele D^2 therefore is below 0.01, so can be compared with the frequency of the allele 6^F of the Est-6-locus in the line RE. It is surprising, however, that the phenotype D0D0 occurs in all lines with almost the same frequency.

The phenotype D0D0 shows no detectable enzyme in the region of the D-bands of the zymogram. It is not possible to differentiate heterozygotes with the hybrid enzyme D1D0 from homozygotes with the isoenzyme D1D1, so their frequency can not be estimated separately. The maximum-likelihood-method for calculating the allele frequencies was used as far as possible to get a better estimation of these frequencies and their variances (see Table 3).

Discussion

Before discussing the results two aspects should be examined more closely: (1) the role of the isoenzymes and the selectivity of their corresponding alleles; (2) the characters of selection.

The role of the isoenzymes and the selectivity of their corresponding alleles

(a) Esterases: The isoenzymes controlled by the Est-6-locus are nonspecific (Wright, 1963); they can be found during the whole life cycle of the individual but are best determined in 1-8 day-old flies. Other isoenzymes are observed in the zymogram together with the Est-6-esterases, but they do not show the same staining intensity when using 1- and 2-naphthyl-acetate as artificial substrate. It is not known whether these isoenzymes can replace the Est-6-esterases in their physiological role. Wright (1963) suggests that the esterases have only a small specificity for artificial substrates, so their importance remains obscure.

Table 2. Observed frequencies of Aph- and Est-phenotypes

Mode of selection (°C)	Line	Aph-phenotypes D1D1 D1D0	D1D2	D2D2	D0D2	D0D0	Est-phenotypes 6F6F	6F6S	6S6S
25	RE	244	2	-	-	10	-	3	253
18	1a	250	-	-	-	6	-	-	256
18	3a*	247	2	-	-	7	-	-	256
28	3b*	248	-	-	-	8	-	-	256
28	1b	248	3	-	-	5	-	-	256

RE reference line
* lines with exchange of food tubes

Table 3. Estimates of the frequencies of the Aph-D- and Est-6-alleles and their variance

Mode of selection (°C)	Line	Frequencies of the Aph-D-alleles D^1	D^2	D^0	Est-6-alleles 6^F	6^S	Variance of the estimates of D^1	D^2	D^0	6^F
25	RE	0.799	0.004	0.197	0.006	0.994	0.00094	0.00001	0.00093	0.00002
18	1a	0.847	-	0.153	-	1.0	0.00051	-	-	-
18	3a*	0.831	0.005	0.164	-	1.0	0.00093	0.00001	0.00092	-
28	3b*	0.823	-	0.177	-	1.0	0.00057	-		-
28	1b	0.853	0.006	0.141	-	1.0	0.00368	0.00001	0.00368	-

The variance of the estimate of the allele 6^F is equal to that of 6^F in the strain RE. The variance of the estimate of the allele D^1 is equal to that of D^0 in the strains 1a and 3b.
* line with exchange of food tubes.

MacIntyre and Wright (1966) reported different heat stabilities for the isoenzymes controlled by the Est-6-locus, but doubted that this could be significant within the range of temperatures used. In this investigation no attempt has been made to test differences in heat sensitivity.

There is little known about the selectivity of the Est-6-alleles. Both alleles are coding for active enzymes with equal activity (MacIntyre and Wright, 1966). Kojima and Yarbrough (1967) and Yarbrough and Kojima (1967) found a frequency dependence with equilibrium frequency of about 0.3 to 0.4 for the allele 6^F. MacIntyre and Wright (1966) also showed frequency equilibria for the Est-6-alleles. They concluded that the equilibrium is a by-product of selection for linked, interacting gene complexes. Within the selected lines investigated in this study, such a balance between the Est-6-alleles does not exist. No frequency equilibrium was observed. The allele 6^S was fixed in all selection lines but not in the reference line RE.

(b) Phosphatases: Occurrence, activity and inheritance of the larval alkaline phosphatases controlled by the Aph-D-locus have been well analysed (Beckman and Johnson, 1964 a,c; Schneiderman et al., 1966; Wallis and Fox, 1968). They are probably tissue specific. (1) Phosphatases from the larval gut of young larvae show an electrophoretic mobility similar to that of the pupal forms; they disappear at the end of the third instar larval period. (2) Phosphatases from larval skin have a strong activity in the third instar, they have a different electrophoretic mobility and can be modified into pupal forms. (3) Phosphatases from pupae appear as soon as the bands from the skin disappear and they persist into the first few hours of adult life (Schneiderman, 1967). The forms (2) and (3) are controlled by the same locus or by two closely linked ones (Wallis and Fox, 1968). In this investigation only the phosphatases of the third instar larvae were examined. These isoenzymes belong to the form (2) and disappear about 9 to 10 hours after prepuparium. They are specific to skin tissue and are only active for a short period within the life cycle, from the beginning of the third instar to emergence of the fly. If we assume that the phosphatases of the forms (2) and (3) are controlled by two distinct, but closely linked, loci (Wallis and Fox, 1968), the time is shortened to less than half during which selection can affect the frequency of the isoenzymes. In this case the stage of the third instar exclusively is essential.

The most striking differences between the alkaline phosphatases and the esterases are the tissue specificity and the developmental stage at which the isoenzymes occur.

(c) Silent alleles: Phenotypes were found which had no detectable enzyme activity controlled by the Est-6-locus. In these cases silent alleles are assumed (Muhs, 1975). In the case of the Est-6-locus only a few individuals have been observed. These individuals were found in inbred but not in selection lines (Muhs, 1975). It seems that the loss of Est-6-esterase enzyme activity is not lethal, but a detrimental effect in individuals without an active Est-6-esterase enzyme can not be excluded. MacIntyre and Wright (1966) reported on a strain lacking detectable Est-6-activity. Johnson (1964) found homozygous deficiency of another esterase activity governed by the Est-C-locus, which did not noticeably affect the viability of the flies.

In the case of the Aph-D-locus several individuals homozygous for the silent allele D^0 have been found in each line. The frequency of this allele was almost the same for all lines. The mechanism responsible for the maintenance of the silent allele D^0 is not known. It can be interpreted in three ways: (1) D^0 reacts in a strictly neutral way concerning selection on temperature; the initial population from which all lines have been derived could be the common source for the almost equal frequencies of the allele D^0 (see Tables 2 and 3). (2) The homozygotes D^0D^0 show a selective disadvantage, the heterozygotes D^0D^1, and D^0D^2 no noticeable disadvantage. In this case the allele D^0 will be eliminated, if no new mutants of the type D^0 are produced. The elimination may be balanced by mutation so that a mutation-selection-equilibrium will be maintained. In fact, there is reason to believe that such an equilibrium exists, since all selection lines have maintained almost the same equilibrium - frequency independent of the mode and intensity of selection. (3) A selective advantage of the heterozygotes D^0D^1 and D^0D^2 may exist during certain stages of development or in some population structures. In this investigation no experiments have been made to calculate fitness values of the different genotypes. Thus selectivity is not proved.

Characters of selection

Selection essentially helps to determine which genotypes have the chance to reproduce. Only reproducing genotypes can influence the population structure of the following generations.

In this investigation the line RE must be regarded as a direct continuation of the initial population. It was always held at 25 °C, the optimal temperature for *Drosophila melanogaster*. Therefore it can be used as reference line compared with the selection lines.

Some general characters of selection, which are mostly common to all selection lines as well as to the line RE, will be examined in detail.

(a) Temperature: The environment of the "cold" lines at 18 °C differs clearly from the optimal temperature of 25 °C. The "warm" lines at 28 °C seem to have an environment which exerts only a small difference compared with the temperature of 25 °C. But these are really extreme conditions: even at a temperature of 30 °C to 32 °C the females do not lay any eggs and the population dies out.

(b) Humidity: It was not possible to find out how far the environment is influenced by other factors. It is conceivable that humidity is important. Humidity can influence the development of the larvae and pupae, e.g. extremely humid food prolonged the duration of larval and pupal development by several days. During summer in the cold incubators (18°C) condensation water was formed and in these cases atmospheric moisture of about 100% was reached. This may have influenced the developmental conditions in these cages.

(c) Overlapping generations: An important and common character of the selected lines is that they are permanently held in big population cages holding about 6000 flies. The generations are not distinctly separated but overlap extensively so that backcrosses can not be excluded. The experimenter does not directly interfere with the process of the selection. Every individual is a potential ancestor of the following generation.

(d) Population density: During the first generations of the initial population there was a rapid expansion of population size. Later, the density of population was regulated by the population itself. The amount of food is a minimizing factor; that means that only a few larvae are able to develop in fresh food.

(e) Competition: The selection is marked by strong competition for food and living space. The competition continues after maturity and determines the success of mating and oviposition. Selection pressure will be high and constant so that competition will be a most important character of selection.

Selection lines

The differences in allelic frequencies had to be interpreted with two questions in mind: (1) What has happened during the time from the foundation of the initial population to the establishment of the selection lines? (2) How have the allelic frequencies changed since establishing the selection lines? Both answers can give an idea of the mechanism responsible for the change in allele frequencies.

(a) Comparison of the founder stocks and the line RE: No information exists about the allele frequencies of the founder stocks concerning the Est-6- and Aph-D-alleles immediately before the foundation of the initial population. But such data may be reconstructed, if we assume that the founder stocks have not changed their allele frequencies greatly. This assumption can be made because the stocks were held under laboratory conditions for several years before the initial population was founded. During that time they may have maintained a balanced polymorphism. In this case the estimated allele frequencies are similar to those before founding the initial population. On the further assumption that all four founder stocks had contributed one quarter to the initial population, the new gene pool of the initial population must have been highly heterogeneous. In terms of allele frequencies this means that all investigated alleles (Aph-D^1, D^2, D^0; and Est-6^F, 6^S) were present, the lowest one with a frequency of 5% or more. From this we may conclude that the newly established gene pool of the initial population must have changed fundamentally, if compared with the allele frequencies of the line RE. Both the Est-6^F and the Aph-D^S-allele exist only at a frequency below 0.01.

The change in the gene pool of the line RE is evident. Indeed fluctuations were observed during the first generations of random mating, which can be explained by heterosis and by a partial breakdown of the following generation. In this time and during the following generations the gene complexes which were adapted for the single stocks were destroyed by recombination. Newly adapted gene complexes were built up for the new gene pool of the line RE. As the selection lines were not derived until the 17^{th} to 19^{th} generation after foundation of the initial population, the allele frequencies of the line RE had probably found new equilibria. This may explain the great similarity in allele frequencies between the line RE and the selection lines.

(b) Comparison of RE and selection lines: The line RE is the only one investigated here which has maintained all alleles. The most frequent alleles are the Est-6^S- and the Aph-D^1-alleles. This result is common to all selection lines. There are only three peculiarities of the line

RE distinguishing it from the other lines: (1) the allele Est-6^F occurred only in line RE, with a small frequency; (2) the allele Aph-D^2 was found at a low frequency in line RE, and has also been observed in two selection lines with equal frequencies (line 3a with exchange of food tubes, 18°C; and line 1b, 28°C); (3) line RE had the highest frequency of the Aph-D^0-allele, which was found in all the selection lines, too, but at somewhat lower frequencies.

We may conclude that in the selection lines selection had merely a small effect. This is true for both the "cold" lines (18°C) and the "warm" lines (28°C). The exchange of food tubes necessarily accompanied by the exchange of genes of the lines 3a (18°C) and 3b (28°C) had no noticeable influence on the distribution of the allele frequencies. All differences between the selection lines including the reference line are not significant.

Three main conclusions can be drawn from these results:

(1) Within the time of selection from the initial population to the date of examination the changes in allele frequency had obviously happened during the first generations of selection. (2) The rather small frequencies of the Est-6^F- and the Aph-D^2 alleles can no longer be regarded as balanced frequencies. Elimination by drift in the more than 125 generations of selection would be conceivable in those cases in which these alleles had been eliminated. How far the distribution of the alleles was influenced by linkage disequilibrium between the Aph-D-locus (on position 3-47.3) and the Est-6-locus (on position 3-36.8) or other genes or gene complexes remains obscure.

(3) The most surprising result it the almost identical frequency of the silent allele Aph-D^0 in all lines. What is a silent allele and how can it be maintained in a population? Can the population fitness be increased by it? At present there are no answers.

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