

Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura*

IV. RELATIVE VIABILITY*

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1. INTRODUCTION

Natural and artificial populations of living organisms change genetically with time. Usually the changes are gradual and slow. However, following an acute irradiation or a drastic environmental change which causes only a small fraction of the population to survive and reproduce, the genetic composition of a population may change drastically within a few generations. When a population is divided into two or more sub-populations, natural selection might operate in different ways in the derived populations. Genetic divergence may occur among such local populations, and may finally lead to race or subspecies formation.

Six experimental populations of *Drosophila pseudoobscura* were begun by Dr M. Vetukhiv in May 1958. They offer an opportunity to study the selective effects of temperature on populations. The populations, obtained by intercrossing strains derived from four different geographic regions, were genetically identical at the beginning. Two populations have been maintained at each of three different temperatures, 16°, 25°, and 27°C., for over 8 years before the present experiments were started. Earlier, Ehrman (1964), Mourad (1965), and Anderson (1966) found that the populations had diverged with respect to mating behavior, longevity, and body size.

Vetukhiv (1953, 1954, 1956) found that hybrids obtained in the laboratory between different geographic populations of *D. pseudoobscura* possess a higher viability in the F₁ generation. This heterosis (or luxuriance) disappears in the F₂ generation, which shows, instead, a breakdown of the viability, sometimes to a level below that not only of the F₁ but even of both parental populations as well. Experiments of Brncic (1954), in which separate chromosomes of different populations were followed with the aid of mutant markers, have shown a similar situation. The F₁ hybrids were more viable than the nonhybrids, while the recombination in F₂ and F₃ led to a breakdown of the viability. The same author (1961) has found an integration of the genotype also in *D. pavani*, manifested by a rapid decrease of the inversion heterozygotes in geographically mixed populations. On the other hand,

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McFarquhar & Robertson (1963), working with geographic populations of *D. subobscura*, found no evidence either of an F₁ luxuriance or of an F₂ breakdown.

In the present study experimental laboratory populations, instead of natural populations, are being used. As stated above, these populations were genetically similar when they were initiated 8 years ago, but they are known to have diverged genetically in some respects. The problem is, then, whether or not this divergence may lead to luxuriance in the F₁ hybrids and to breakdown in later hybrid generations. Pre-adult viability, the character studied, is one of the important components of fitness.

2. MATERIALS AND METHODS

The temperatures at which the six experimental populations were maintained were: A and B at 16°C., C and D at 25°C., and E and F at 27°C. All six populations are descended from the same founders, hybrids of Arrowhead homokaryotypes, derived from flies collected in four different localities in California, Utah and Colorado. For further details on Vetukhiv's populations, see Ehrman (1964).

A scheme of the experimental crosses is shown in Fig. 1. In July 1966, *Ba/Δ*; *L* females were mated with *Cy* males. In Generation 6 (G-6 in Fig. 1), twenty-two

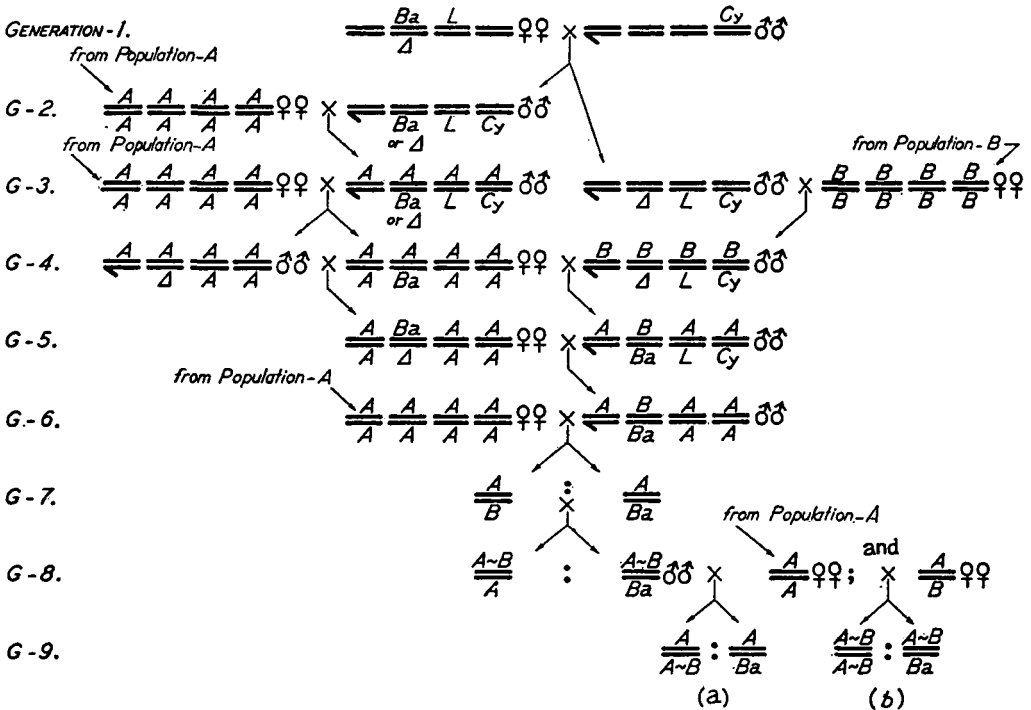


Fig. 1. A scheme of the crosses used in the experiments. The four large chromosomes of *Drosophila pseudoobscura* are represented schematically. *Ba*, Δ , *L* and *Cy* are the mutant markers; A and B symbolize the chromosomes derived from different populations; the sign : marks the generations in which counts of the flies were made. The sign ~ indicates the recombined A-B chromosomes. Twenty-two experiments, involving different combinations of the six experimental populations, have been made.

different experimental stocks were established, in which the four major chromosomes of the six original populations were substituted by means of the dominant marker-inversion technique. The genotypic constitution for the second chromosomes and the other major chromosomes, e.g. the genetic background of these stocks shown in parentheses, are indicated as follows: A/Ba(A)—this means that the second chromosomes are heterozygous for the wild-type chromosome derived from the population A and for the mutant *Bare*, with a population A genetic background. The other stocks are as follows: B/Ba(B), C/Ba(C), D/Ba(D), E/Ba(E), F/Ba(F), B/Ba(A), C/Ba(A), A/Ba(B), D/Ba(B), F/Ba(B), A/Ba(C), D/Ba(C), B/Ba(D), C/Ba(D), A/Ba(E), C/Ba(E), F/Ba(E), B/Ba(F), D/Ba(F), and E/Ba(F). In each generation during the course of the chromosome substitution approximately fifty females for each cross were used to serve as the parents. This was done in order to avoid inbreeding in the experimental stocks and to maintain a genetic diversity more or less representative of the parental populations. Two stocks E/Ba(A) and E/Ba(C), were lost in the process of the chromosomal substitution. In a few other cases the number of second chromosomes was reduced in some stocks to about ten in some generations; however, generally most of the experimental stocks contained numbers of second chromosomes large enough to be representative of each original population.

In Generation 6, virgin females were sampled from the original populations, and twenty-two further crosses were made, as shown in Fig. 1. The preadult viabilities were measured by the ratios of the wild-type flies to *Ba* in the cultures. Each kind of cross was made in triplicate, and the parental flies were transferred into fresh bottles three to four times. Then, three or four replicate cultures for each combination were raised at each of three different temperatures (16°C., 25°C., and 27°C.). Half-pint bottles with Spassky's cream of wheat-molasses medium inoculated with yeast were used. Complete counts of the flies emerging were made. In Generation 7, enough virgin females, more than ten per cross, which were heterozygous for wild-type second chromosomes derived from two different populations, were collected for making the crosses with *Ba* males recovered from the same bottles (Fig. 1). In Generation 8, the flies were counted again. Males derived from the cultures at 25°C. in Generation 7, whose second chromosomes were heterozygous for *Ba* and for the recombinants between the chromosomes derived from different populations, were crossed with two kinds of females in separate bottles. One kind of the females was taken directly from the original populations. The other kind of females, which had two different (nonrecombinant) second chromosomes in heterozygous condition, were taken from the last bottles of the same combinations at 25°C. in Generation 6. The same procedures were used for four kinds of crosses in the Generations 6 to 8, as shown in Fig. 1. At 16°C. flies with the *Ba* chromosome frequently had bristles close to the normal phenotype. Misclassification of these flies was avoided by careful inspection.

3. RESULTS

Table 1 shows the percentages of wild-type flies observed in the six kinds of intrapopulation crosses, i.e., A/A(A) vs. A/Bare(A), B/B(B) vs. B/Bare(B) . . .

Table 1. Percentages of wild-type flies in the control experiments, carrying chromosomes from a single population only. *N* is the number of the flies counted

Temperatures	Populations					
	A	B	C	D	E	F
16°C.	50.637	48.497	51.455	49.031	49.182	50.233
	49.306	50.902	49.793	51.117	49.769	49.423
	50.000	50.420	49.300	50.737	48.076	50.120
	50.487	49.833	48.890	48.771	48.910	48.614
	<i>N</i> = 8422	<i>N</i> = 7735	<i>N</i> = 9340	<i>N</i> = 7121	<i>N</i> = 11,401	<i>N</i> = 9482
	\bar{X} = 50.3700	\bar{X} = 49.7436	\bar{X} = 49.7907	\bar{X} = 49.8569	\bar{X} = 48.8960	\bar{X} = 49.3453
25°C.	49.797	50.656	51.124	50.240	49.817	50.000
	48.332	50.528	51.648	49.824	48.387	50.620
	50.288	49.120	52.673	50.094	50.000	51.429
	48.362	50.032	50.938	51.601	48.170	48.296
	<i>N</i> = 7003	<i>N</i> = 5737	<i>N</i> = 7698	<i>N</i> = 7174	<i>N</i> = 7999	<i>N</i> = 8150
	\bar{X} = 48.9750	\bar{X} = 50.1831	\bar{X} = 51.3771	\bar{X} = 50.6947	\bar{X} = 49.2557	\bar{X} = 50.1147
27°C.	49.243	48.107	50.539	51.615	50.496	49.641
	48.241	49.060	50.840	50.318	48.779	49.476
	48.234	48.721	49.963	50.574	48.855	50.909
	47.851	47.505	48.128	50.394	48.506	50.780
	<i>N</i> = 5426	<i>N</i> = 4367	<i>N</i> = 5136	<i>N</i> = 5041	<i>N</i> = 6335	<i>N</i> = 5184
	\bar{X} = 48.3469	\bar{X} = 48.1900	\bar{X} = 49.8075	\bar{X} = 50.7342	\bar{X} = 49.2623	\bar{X} = 50.2285

F/F(F) vs. F/Bare(F). The letters A, B, . . . F outside the parentheses indicate the second chromosomes, and those in the parentheses the genetic backgrounds. The four lines in each column are the results obtained in experiments corresponding to the generations G-7 to G-9 in Fig. 1. The means, \bar{X} , are the unweighted averages of the four lines for each kind of cross. An analysis of variance has been made. The results show that the variance between the populations, and the interaction between the populations and temperatures, are significantly different at 0.01 and 0.05 levels, respectively. Although the frequencies of the wild-type class are close to 50%, combining the pairs of populations which were raised at the same temperatures, the viabilities tend to be highest at the temperatures at which the respective populations were maintained for the 8 years preceding the experiments. The percentage frequencies in Table 1 serve as the control values for further comparisons.

First, we wish to compare the viabilities of the flies having both second chromosomes from the same population which provides the genetic background with that of the flies carrying one foreign second chromosome, i.e., A/A(A) vs. A/B(A), B/B(B) vs. B/A(B) . . . F/F(F) vs. F/E(F). The results are shown in Fig. 2. The zero level line corresponds to the intrapopulation wild-type frequencies listed in Table 1. The white columns in Fig. 2 indicate the differences between the respective inter-population crosses and the intrapopulation heterozygotes. Out of the forty-eight such comparisons, only three show the white columns below the zero level line,

and in none significantly so. On the other hand, eighteen out of the remaining forty-five comparisons show the viability of the interpopulational heterozygotes significantly above the control level. These eighteen are shown in Fig. 2 by heavy lines. They are also listed in Table 2 in the column headed 'Comparisons 1'. This is, evidently, an indication of a heterotic effect of the interpopulational heterozygosis.

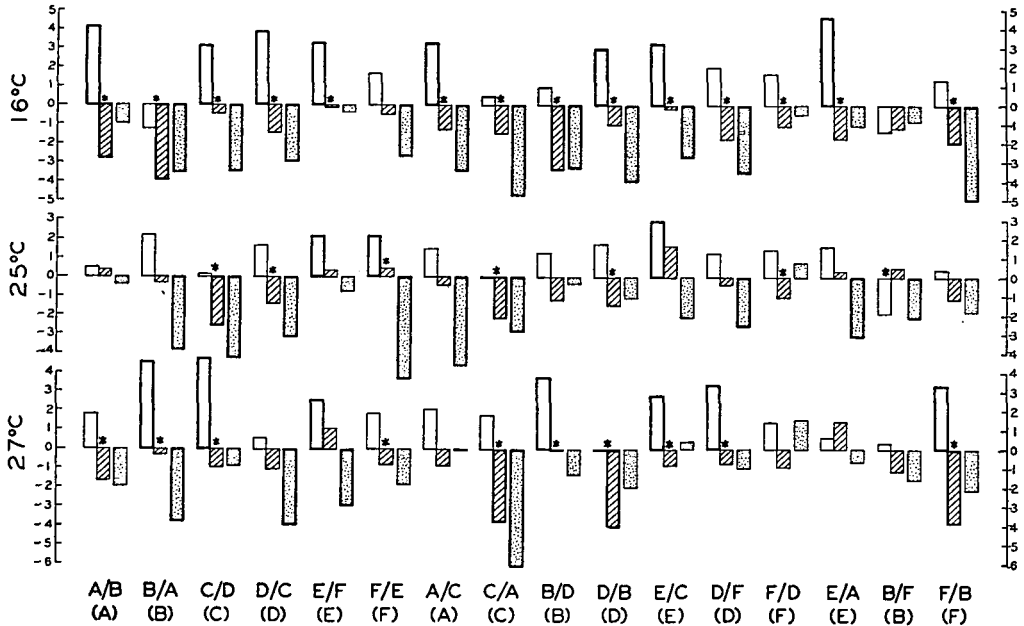


Fig. 2. Gain or loss of the viability resulting from hybridization. The zero (control) level refers to the viabilities (measured in percentages of wild-type flies in the cultures) of flies carrying chromosomes of only a single population; the white columns indicate the differences between the controls and the flies carrying one non-recombined foreign chromosome; the hatched columns show the differences between the controls and the heterozygotes for recombinant chromosomes; the dotted columns give the differences between flies carrying one recombinant chromosome and those having both chromosomes of a pair resulting from recombination.

A second comparison is between the intrapopulational control values and the values observed in flies carrying one second chromosome agreeing with the genetic background and the other second chromosome being a result of recombination of second chromosomes from two populations, i.e., A/A(A) vs. A/A ~ B(A), B/B(B) vs. B/B ~ A(B) . . . F/F(F) vs. F/F ~ E(F). The recombinant chromosomes are obtained in G-9a generation shown in Fig. 1. In Fig. 2 the results are shown by the hatched columns. In only seven out of forty-eight comparisons are the hatched columns above the control level, never statistically significantly so. Of the forty-one remaining comparisons, nine are significantly below the control. The significant combinations are shown in Table 2 by the asterisks in the column headed 'Comparisons 2'.

A third comparison is between the interpopulational heterozygotes (G-7 in Fig. 1) and the recombinant heterozygotes (G-9a in Fig. 1). As shown in Table 2, in the column headed 'Comparisons 3', in thirty-one out of forty-eight comparisons the viabilities are significantly different. In thirty out of these thirty-one the recombinant heterozygotes are less viable than the nonrecombinant ones. The one exception occurs in the hybrids between the populations B and F (B genetic background), in which the heterozygote in G-7 is ostensibly below the control level, though not significantly so. In Fig. 2 the significant differences are indicated by the asterisks.

Table 2. *Numbers of statistically significant differences observed in the four kinds of comparisons. Further explanation in text*

Genetic background	Combination of the second chromosomes	No. of significant cases	Comparisons (°C.)													
			1			2			3			4				
			16°	25°	27°	16°	25°	27°	16°	25°	27°	16°	25°	27°		
A	A/B	4	*			*			*		*					
A	A/C	4	*						*				*	*		
B	B/A	7			*	*			*		*	*	*	*	*	*
B	B/D	5			*	*			*		*	*	*			
B	B/F	2								*				*		
C	C/A	8					*	*	*	*	*	*	*	*	*	*
C	C/D	8	*		*		*		*	*	*	*	*	*		
D	D/B	6	*					*	*	*	*	*	*			
D	D/C	6	*						*	*			*	*	*	
D	D/F	5			*				*		*	*	*	*		
E	E/A	3	*						*						*	
E	E/C	6	*	*	*				*		*	*	*			
E	E/F	5	*	*	*				*		*	*	*			*
F	F/B	6			*	*			*		*	*	*			
F	F/D	2							*	*						
F	F/E	5		*						*	*	*	*	*	*	
			.8	3	7	4	2	3	14	7	10	11	9	4		
		82/192	18/48			9/48			31/48			24/48				

A fourth comparison is between the flies carrying one recombinant chromosome (G-8 in Fig. 1) and those carrying two recombinant chromosomes (G-9b). That is to say, we compare A/A ~ B(A) with A ~ B/A ~ B(A), B/B ~ A(B) with B ~ A/B ~ A(B) . . . F/F ~ E(F) with F ~ E/F ~ E(F). Table 2 shows that twenty-four out of forty-eight comparisons show statistically significant differences (column headed 'Comparisons 4'). In Fig. 2 the frequency of the wild-type class in the G-8 generation cross is taken as the base line, and the G-9b results are shown by the dotted columns. In only three out of forty-eight comparisons are the dotted columns above the base lines, and in twenty-four out of the remaining forty-five significantly below.

In the four series of comparisons a total of 452,632 flies were counted, 112,030,

107,385, 113,443 and 119,774 in the Generations G-7, G-8, G-9a and G-9b respectively. Following Brncic (1954), chi-square tests were applied in each pair in all the comparisons.

4. DISCUSSION

The fitness of hybrids between populations or races of the same species is apparently not subject to any consistent rule. Thus Hulse (1957) found in Switzerland that persons whose parents came from different villages are significantly taller on the average than those whose parents came from the same village. Yet the detailed study of Morton, Chung & Mi (1967) in Hawaii failed to disclose any evidence either of heterosis or of hybrid breakdown in the progenies of inter-racial marriages in Hawaii. Vetukhiv (1963, 1954, 1956) studied the egg-to-adult viability, fecundity and longevity of hybrids between populations of different geographic origins in *D. pseudoobscura*, *D. willistoni*, and *D. paulistorum*. In *D. pseudoobscura* and *D. willistoni* the F₁ hybrids are superior to their parents, but the F₂ hybrids not only lose the heterosis but most of them are in fact inferior to the parental populations. In *D. paulistorum* such a hybrid breakdown has been observed in only one of the three crosses examined. No evidence of either an increase or a decrease in the components of fitness was found in the hybrids between populations of *D. subobscura* studied by McFarquhar & Robertson (1963).

Brncic (1954) and Wallace & Vetukhiv (1955) analyzed the situation in more detail, by making use of mutant markers which permitted them to study the effects of separate chromosomes. In the experiment of Brncic on *D. pseudoobscura* the flies which carried two third chromosomes of different geographic origin were more viable than the flies with two third chromosomes from the same geographic locality. He also observed a breakdown of the viability in F₂ and F₃ generations. He concluded that the establishment of harmoniously integrated genetic systems is not characteristic of species but of local populations. Wallace and Vetukhiv compared the egg-to-adult viability in progenies with different degrees of heterozygosity, using irradiated populations of *D. melanogaster* and natural populations of *D. pseudoobscura*. The viability was found to be increased by heterozygosity but decreased by recombination within and between chromosomes from different populations.

In the experiments described in the present article, laboratory populations of known origin, instead of natural populations, were used. The six populations were all descended from quadruple hybrids between populations of four different localities in western United States. The same group of founders were used to deposit the eggs in the six experimental populations. There were two replicate populations kept at each of the three temperatures: populations A and B at 16°C., C and D at 25°C., and E and F at 27°C. The temperature 16°C. is closest to the optimum for the species, but at 25°C. the flies breed easily. At 27°C. the populations were at first difficult to maintain, and during the first 2 years of their existence (when they were taken care of by Dr M. Vetukhiv and by Mrs Olga Pavlovsky) they

had to be occasionally given temporary respite by being transferred to 25°C. for a generation. Eventually they became vigorous enough to be maintained exclusively at 27°C., although the numbers of the flies in these populations are always smaller than in those kept at the lower temperatures.

Pre-adult viabilities were chosen as the trait to be measured, because this trait is one of the most sensitive components of fitness affected by the genotypic constitution and also by environmental factors. The results of the present experiment show indications of genetic divergence among the populations. Variance analysis shows significant differences between the populations, and also first-order interactions between temperatures and populations. The populations have the highest relative viabilities at the temperatures at which they have been kept (Table 1). The average viability of all six populations considered together is the same at the three temperatures, since the lowered viability of some of them is compensated for by the higher viability of others.

There are several indications of genetic divergence among the six populations (see Table 2). In the comparison (1), eighteen cases out of forty-eight show F_1 luxuriance compared with the original parental genotypes (intrapopulational combinations). In the comparison (2), nine out of forty-eight have significantly lower viabilities compared with the parental genotypes, due to recombination which occurred in Generation 8 (cf. Fig. 1); nine out of forty-eight is not so high an incidence, but it is reasonable because not all chromosomes are recombinants in Generation 9. The comparison (3), between F_1 and the recombinants, shows much more striking differences than the comparisons (1) and (2). The comparisons (2) and (3) are concerned with the effects of recombination in the interpopulational heterozygotes. The comparison (4) also serves to reveal the effects of recombination between chromosomes derived from different populations. Wild-type flies obtained in Generation 9b have the second chromosomes both of which are of recombinant origins (Fig. 1). One half of the forty-eight cases show significant decreases in the relative viability.

To review the incidence of significant differences in all four comparisons at all temperatures, the results are summarized in Table 3. A fact which may seem surprising at first sight is that significant differences are observed not only between populations which were living for 8 years at different temperatures but also between those which have been kept at the same temperature (marked by asterisks in Table 3). What this means is presumably that a genetic divergence can occur not only between populations exposed to different environments but also between those living in similar environments. Gene pools which emerge as a result of natural selection in a given environment need not be the same in different independently selected populations. Isolated natural, as well as experimental, populations may come to differ genetically, and these differences may be coadaptive, giving a loss of fitness when the populations are crossed and recombination is allowed to occur. The heterosis in the F_1 hybrids depends, presumably, on the degree of homozygosity in the populations crossed. The loss of fitness in F_2 and F_3 is more readily explained than the luxuriance or heterosis in F_1 . If the populations have different coadapted

Table 3. *Numbers of significant differences in various combinations of populations; the numbers in parentheses indicate the total combinations studied*

Combination of the second chromosomes	Number of significant differences
A/B	11* (24)
A/C	12 (24)
B/D	11 (24)
B/F	8 (24)
C/D	14* (24)
D/F	7 (24)
E/A	2 (12)
E/C	6 (12)
E/F	10* (24)

* Populations kept at the same temperature.

genetic systems, these systems may break down, and may lead to a lowering of the fitness, until new coadapted systems are built by selection in the populations of hybrid origin. Neither luxuriance nor breakdown are however expected if the populations have similar coadapted systems. In this regard, the difference in the outcomes of the hybridization experiments between populations of the fairly closely related species, *D. pseudoobscura* and *D. subobscura*, is very interesting. As mentioned above, McFarquhar & Robertson (1963) found no indication of either luxuriance or hybrid breakdown in the hybrids between populations of the latter species. This may well be related to the different behavior of the chromosomal polymorphisms in these two species discovered by Anderson, Dobzhansky & Kastritsis (in press) and by Krimbas (in press). The equilibrium frequencies of the chromosomal polymorphs in experimental populations of *D. pseudoobscura* are often quite different from the natural populations from which these polymorphs were derived. In *D. subobscura* such differences are much less pronounced. The gene pool of the former species seems to be 'flexible' and of the latter species 'rigid' (Dobzhansky, 1962).

SUMMARY

Six experimental populations of *D. pseudoobscura* were maintained at three different temperatures for more than 8 years. All the populations have originally descended from the same founders. The populations were examined for evidences of genetic divergence. The pre-adult viabilities of the flies from these populations, and of their F₁ and F₂ hybrids, were studied at 16°C., 25°C., and 27°C. The results indicate that the populations have diverged genetically from each other. Some divergence is observed not only between populations kept at different temperatures, but also between the replicate populations kept at the same temperature.

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