GENETICS OF NATURAL POPULATIONS. XXXIX. A TEST OF THE
POSSIBLE INFLUENCE OF TWO INSECTICIDES ON THE
CHROMOSOMAL POLYMORPHISM IN
*DROSOPHILA PSEUDOOBSCURA*¹

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NATURAL populations of *Drosophila pseudoobscura* are polymorphic for the gene arrangements, particularly in the third chromosomes. The relative frequencies of the different karyotypes are sensitive to the environmental conditions, and may change seasonally from month to month, as well as from year to year. During approximately a quarter of a century, 1940–1964, very significant changes in the chromosomal composition of the populations have taken place, especially in the Pacific Coast states, from California to British Columbia (DOBZHANSKY, ANDERSON and PAVLOVSKY 1966, and further references therein). The causation of these year-to-year changes remains an unsolved problem. Attempts to correlate the genetic changes with climatic variations, such as droughts and rainy years, were not successful. Correlations with biotic variables, such as relative frequencies of competing *Drosophila* species, were also absent (DOBZHANSKY, ANDERSON, PAVLOVSKY, SPASSKY and WILLS 1964). One is led to look for a possible explanation in environmental changes caused by man. The genetic changes in the population of a California locality (Mather) were first detected in the late nineteen-forties. This happens to coincide with the beginning of mass use of DDT as an insecticide for controlling agricultural pests in California. Although *D. pseudoobscura* lives chiefly outside the agricultural zones in which the insecticides are applied on a large scale, traces of insecticide contaminants may be found well outside these zones. It is, then, conceivable that the carriers of some karyotypes of *Drosophila* are more, and of others less resistant to these contaminants. If so, the populations may be altered genetically by the insecticides. Experimental populations were first used to test this hypothesis by OSHIMA and WATANABE (1965, 1966) at the National Institute of Genetics, Misima, Japan. The results, though inconclusive, did not rule out the possibility that some chromosomal variants differ from others in insecticide resistance. Experiments were, accordingly, continued by OSHIMA and WATANABE in Japan, and other experiments, utilizing slightly different techniques, were made by PAVLOVSKY and DOBZHANSKY in New York. The results are reported in the present article.

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MATERIALS AND METHODS

Strains of *D. pseudoobscura* with the dominant genes Blade and Scute, or with Lobe in their third chromosomes, were crossed and backcrossed for several generations to a population descended from flies collected at Mather, California. A *BlSc/L* "analyzer strain" with the chromosomes other than the third replaced by those of Mather origin was thus obtained. This analyzer strain was then used to extract, with proper cytological controls, strains with known gene arrangements in the third chromosomes from a fresh sample of flies from the Mather locality. Ten strains homozygous for each: Standard (ST), Arrowhead (AR), Tree Line (TL); twenty-two Pikes Peak (PP); and seven strains homozygous for the Chiricahua (CH) gene arrangements were thus prepared. Because of the extraction technique used, these strains were originally free of recessive lethal, semilethal, and sterility genes in the third chromosomes, which occur in any sizeable sample of wild chromosomes.

The four gene arrangements ST, AR, CH and PP, were involved in the experiments made in Japan. AR females from all the strains were crossed to ST males, and CH females to PP males. The AR/ST heterozygous females were then crossed to CH/PP males; 250 females and 250 males were taken in the next generation and, in August 1964, introduced into each of the two population cages, to serve as progenitors of experimental populations. The progenies hatching in each cage were divided in two parts. One was exposed in a plastic test cylinder for 1 hour to paper impregnated with 2 per cent solution of DDT (3.6 mg/cm², supplied by the World Health Organization in Geneva); the other part was untreated; these flies were the progenitors of the Control I and a DDT-treated populations. Four similar batches of flies served to initiate two populations (DLR-I and DLR-II) exposed for one hour to papers impregnated with 0.1 per cent of Dieldrin and two corresponding control populations (Control II and Control III). Once in three weeks, the adult flies hatching in the population cages (and a few surviving parents) were taken, treated with DDT or with Dieldrin, or transferred without treatment in the control populations. Roughly 1500 flies were the progenitors of each generation (the Control III and DLR-II populations were started later than the others, in April 1965). This technique makes the initial chromosome frequencies different in the three controls and the three treated populations paired with them (Generation S-O, Table 1).

The experiments in New York involved five gene arrangements: ST, AR, CH, PP, and TL. Crosses were made in the following combinations: PP × TL, PP × AR, ST × CH. ST × TL and AR × CH (22 PP strains were used in this experiment). The following numbers of the hybrids were then placed in a population cage (December 1964):

TL/PP	1200	ST/TL	600
AR/PP	800	AR/CH	600
ST/CH	800	Total	4000

At 2-3 days intervals, sets of 15 cups with culture medium were inserted in the cage, and when the surface of the medium was covered with eggs the cups were withdrawn, transferred to six new cages without adults, and allowed to develop. The initial gametic chromosome frequencies were accordingly the same in all cages, namely:

PP — 25.0%	AR — 17.5%
TL — 22.5%	CH — 17.5%
ST — 17.5%	100%

Two of the cages were controls, two were treated with DDT, and two with dieldrin in each generation. The duration of the treatments was 4 hours (instead of 1 hour in Japan), but the impregnated papers used had 0.1% or 0.01% concentrations for DDT, and 0.05% and 0.005% for dieldrin. In general, 3 test cylinders with greater and 3 with lesser concentration were used in each generation, and the numbers of the flies treated on paper with higher and with lower concentrations of the insecticides were approximately equal. Though the duration of the treat-

ments in the American experiments was four times longer than in the Japanese ones, the insecticide concentrations were much higher in Japan. In the American experiments the mortality of the treated flies was minimal; it never exceeded 10 per cent of the individuals treated. In the Japanese experiments the mortality was about 20 per cent after exposure to 1 per cent DDT test paper and about 35 per cent after exposure to 0.1 per cent Dieldrin test paper at the beginning of the experiment. Females and males sampled separately from Control I and DDT selected populations (S-28), just before the end of the experiment, were exposed to 4 per cent DDT test paper for 30 minutes and after 24 hours the mortalities were determined by three times replicated tests. The mortality of the selected population was 34.8% in females and 38.7% in males and that of the Control population was 71.1% in females and 71.8% in males. Consequently, the resistance to DDT of the selected population was higher than that of the nonselected population.

In every generation at the beginning, and once in two or more generations later in the experiments, the chromosome frequencies were studied in all the populations. Samples of the eggs deposited in the population cages were taken, the larvae were grown under optimal conditions, and the gene arrangements in 300 third chromosomes (150 larvae) were determined in squash preparations stained with acetic or lactic orcein, except as follows: Control-III, S-3 generation—280 chromosomes, DLR-II, S-4 generation—228 chromosomes (the experiments in Japan), and all samples in the S-1 generation, experiments in New York—100 chromosomes.

RESULTS

The Course of Selection: The gametic frequencies of each kind of chromosomes found in the samples in different generations are shown in Table 1 (experiments in Misima, Japan) and in Table 2 (experiments in New York). In every population without exception, the ST chromosomes increased in frequencies, and the CH, PP, and TL chromosomes dwindled, in some populations ostensibly to zero. AR behaved quite differently in the American and the Japanese experiments; in the former it always increased in frequency, and by the eleventh generation of selection (S-11 in Table 2) overtook the ST. In the Japanese experiments AR always became much less frequent than ST, and in DLR-I ostensibly disappeared in S-18; only in Control-I it remained common, but less so than ST (Table 1). In fact, the Japanese populations, with the exception of Control-I, became nearly monomorphic ST, while the American ones continue polymorphic for ST and AR, and usually conserve some PP, and TL chromosomes as well.

Statistical Analysis: Several methods of estimation of the adaptive values of chromosomal types in experimental populations of *Drosophila* have been suggested. WRIGHT (in WRIGHT and DOBZHANSKY 1946) estimated adaptive values with a least squares procedure. CAVALLI (1950) developed maximum likelihood estimates for populations with two heterotic alleles, using a model of continuous change. LEVENE (in LEVENE, PAVLOVSKY, and DOBZHANSKY 1954) used the method of minimum chi-square. We have used a maximum likelihood method to obtain estimates of the adaptive values. Full details of our procedure will be given elsewhere (DUMOUCHEL and ANDERSON 1968). A sketch of the essentials of the technique is as follows.

Estimation by the method of maximum likelihood is preferable for several reasons. It leads to estimates which maximize the probability of the particular observations which were obtained. Maximum likelihood estimation yields the sampling variances and covariances of the estimates, which give an idea of their

TABLE 1

Frequencies, in per cent, of four kinds of chromosomes in treated and control populations (Experiments in Misima)

Chromosomes	S-0	S-1	S-2	S-3	Generations		S-10	S-13	S-18	S-25
					S-5	S-7				
Control I										
ST	33.3	42.0	45.7	53.7	65.3	72.3	67.0	65.0	52.7	63.7
AR	26.3	24.7	25.7	26.3	22.0	17.0	27.0	34.3	46.7	36.3
CH	22.7	18.3	17.3	10.0	7.0	6.3	4.7	0.7	0	0
PP	17.7	15.0	11.3	10.0	5.7	4.3	1.3	0	0.7	0
DDT										
ST	33.3	40.3	55.0	55.3	73.3	77.0	92.7	86.3	87.0	94.3
AR	26.3	18.3	23.0	24.3	15.7	18.7	6.3	7.7	9.7	5.3
CH	22.7	22.0	11.7	8.3	4.0	0.7	0	0	0	0
PP	17.7	19.3	10.3	12.0	7.0	3.7	1.0	6.0	3.3	0.3
	S-0	S-1	S-2	S-3	S-5	S-7	S-10	S-16	S-18	
Control II										
ST	26.3	36.3	45.7	49.3	52.3	64.3	68.7	94.7	94.0	
AR	28.0	21.7	29.7	27.3	30.0	25.3	26.3	5.3	5.7	
CH	26.3	23.3	14.7	13.3	12.0	5.3	3.0	0	0	
PP	19.3	18.7	10.0	10.0	5.7	5.0	2.0	0	0.3	
DLR-I										
ST	26.3	43.7	41.0	43.0	71.0	84.3	90.3	96.0	98.7	
AR	28.0	19.7	30.0	29.7	16.7	5.7	2.0	1.3	0	
CH	26.3	15.3	9.7	10.3	4.3	6.0	4.7	1.0	0	
PP	19.3	21.3	19.3	17.0	8.0	4.0	3.0	1.7	1.3	
	S-0	S-1	S-2	S-3	S-4	S-8	S-11	S-14	S-17	
Control III										
ST	40.0	41.3	59.0	63.6	73.7	87.3	90.3	90.3	95.7	
AR	40.0	43.3	33.7	30.4	20.7	11.0	8.7	9.3	4.3	
CH	17.0	14.0	6.3	2.5	3.7	1.3	1.0	1.0	0	
PP	3.0	1.3	1.0	3.5	2.0	0.3	0	0	0	
DLR-II										
ST	40.0	48.0	52.7	59.3	73.2	93.0	90.3	94.6	97.7	
AR	40.0	32.7	36.7	32.0	19.7	3.0	4.7	4.7	1.7	
CH	17.0	16.3	6.3	1.7	0.9	0.3	0	0	0	
PP	3.0	3.0	4.3	7.0	6.1	3.7	5.0	0.7	0.7	

reliability. The sampling variances we obtain are those which are approached asymptotically as the sample size increases. The samples were large enough to assume that the theoretical sampling variances describe the variation in our samples, at least to a good approximation.

Estimates of the adaptive values were generated under models of changing inversion frequencies governed by constant selective values. The basic formula in the models is the recurrence relation:

$$p_i(t+1) = \frac{p_i(t) \cdot \sum_j p_j(t) w_{ij}}{\sum_i \sum_j p_i(t) p_j(t) w_{ij}}$$

TABLE 2

Frequencies, in per cent, of five kinds of chromosomes in treated and control populations (Experiments in New York)

Chromosomes	S-0	S-1	S-2	S-3	Generations		S-7	S-9	S-11	S-13
					S-4	S-5				
Control I										
ST	17.5	27	24.3	40.3	47.3	45.0	45.7	37.7	37.3	45.0
AR	17.5	18	18.7	26.3	33.7	34.3	49.7	58.0	60.3	53.3
CH	17.5	10	14.0	6.7	6.7	7.7	0.7	1.0	0.3	0
PP	25.0	21	23.0	17.0	8.0	8.3	3.0	2.0	1.3	0.7
TL	22.5	24	20.0	9.7	4.3	4.7	1.0	1.3	0.7	1.0
Control II										
ST	17.5	21	29.0	24.7	35.3	37.3	43.0	38.3	34.7	38.7
AR	17.5	25	25.7	31.0	35.3	47.0	50.7	60.7	62.7	59.7
CH	17.5	17	18.3	15.3	13.3	3.3	2.7	0.3	0.3	0.7
PP	25.0	21	13.7	15.0	7.7	8.0	2.3	0.7	0	0.3
TL	22.5	16	13.3	14.0	8.3	4.3	1.3	0	2.3	0.7
DDT-I										
ST	17.5	22	24.7	33.7	45.3	45.0	48.0	44.7	46.0	42.0
AR	17.5	20	32.0	29.7	35.3	35.0	42.3	43.0	51.0	53.7
CH	17.5	17	11.7	12.3	7.3	7.3	2.0	7.7	1.3	0.7
PP	25.0	20	18.3	13.3	5.7	10.3	6.3	2.7	1.0	1.7
TL	22.5	21	13.3	11.0	6.3	2.3	1.3	2.0	0.7	2.0
DDT-II										
ST	17.5	21	33.0	41.7	44.7	40.7	50.0	47.3	42.0	42.0
AR	17.5	20	28.0	31.3	33.7	42.3	38.0	44.3	56.0	55.0
CH	17.5	17	11.3	7.0	3.7	4.7	3.3	2.7	0.3	0
PP	25.0	19	14.0	11.3	10.3	7.7	5.0	4.0	1.3	2.0
TL	22.5	23	13.7	8.7	7.7	4.7	3.7	1.7	0.3	1.0
DLR-I										
ST	17.5	20	25.0	27.0	33.7	34.0	50.0	42.7	39.3	42.3
AR	17.5	15	31.0	35.7	33.7	41.7	41.0	48.0	56.0	56.3
CH	17.5	22	17.3	7.7	7.0	2.7	1.3	0.3	0	0
PP	25.0	24	13.3	20.7	18.0	16.0	6.7	7.3	3.3	1.0
TL	22.5	19	13.3	9.0	7.7	5.7	1.0	1.7	1.3	0.3
DLR-II										
ST	17.5	17	24.0	29.7	38.7	31.7	35.0	36.7	32.3	39.0
AR	17.5	21	31.7	28.3	34.0	41.7	54.7	57.3	64.0	56.3
CH	17.5	16	12.7	14.0	5.7	8.7	1.0	0.3	0.3	0
PP	25.0	23	17.3	16.7	16.7	13.3	4.0	1.7	1.7	2.0
TL	22.5	23	14.3	11.3	5.0	4.7	5.3	4.0	1.7	2.7

where $p_i(t)$ is the frequency of the i -th gene arrangement in the generation t , and $w_{ij} \equiv w_{ji}$ is the adaptive value of the genotype containing the chromosomes i and j . The summations in i and j are over the number of types of chromosomes. In our experiments the generations were non-overlapping.

The multinomial distribution of inversion frequencies was used to set up the likelihood equations. These equations, which define the maximum likelihood estimates of the adaptive values, cannot be solved directly. We have therefore

used iterative techniques to obtain close approximations to the true values. The computations were performed with the aid of the IBM 7090-7094 computer system at Yale University. Beginning with a set of trial values, we obtained improved estimates. These were substituted into the likelihood equations, and the procedure yielded estimates progressively closer to the true values. The process was continued until the difference between the successive sets of adaptive values became negligible. We have found that many sets of estimates can be derived which fit the data almost equally well, when as many as four or five inversions are involved. We have included in our estimation a procedure for selecting that set of estimates whose elements are positive within the usual limits of sampling error. Negative adaptive values are, of course, biologically impossible.

The procedure of obtaining estimates gave, for each population, a vector of adaptive values and a matrix of their variances and covariances. These variances and covariances are influenced by our choice of non-negative estimates. We have assumed that the estimates of the adaptive values are multinormally distributed, and compared the populations by forming a vector of differences between the adaptive values. The covariance matrix of this difference vector is the sum of the covariance matrices of the two vectors of the adaptive values. It may then be shown that if all the elements of the difference vector have true mean values of zero, then the following product of matrices has a chi-square distribution:

$$DC^{-1}D' = \chi_n^2$$

where D = difference vector

C^{-1} = inverse of the covariance matrix of the difference vector

D' = transpose of the difference vector

n = number of independent adaptive values in each population.

This chi-square value with n degrees of freedom may be referred to a table to test the significance of the difference between the two sets of adaptive values from the two populations.

There are several considerations one must keep in mind in interpreting the results shown in Tables 3 and 4. With five kinds of chromosomes, there are fifteen adaptive values to be estimated simultaneously for each population. Each estimate can hardly be precise when so many different ones are being extracted from a limited amount of data. The covariance matrices are quite bulky, and we have therefore not included them in the tables of this paper. The interested reader is referred to the paper by DuMOUCHEL and ANDERSON for examples. Typical adaptive values and their standard errors (from Control I of the Misima experiments) are presented below to give the reader a feeling for the variation:

ST/ST	ST/AR	ST/CH	ST/PP	AR/AR	AR/CH
0.69±.13	1.00±.21	0.80±.22	0.72±.22	0.54±.16	0.19±.37
	AR/PP	CH/CH	CH/PP	PP/PP	
	0.31±.47	0.54±.78	0.47±1.15	0.59±1.23	

Despite the fairly large standard deviations, the estimates of the adaptive values are not worthless, since comparisons of whole sets of adaptive values obtained from different populations are still possible. The outcome depends in large part on the *pattern* of variability of the estimates, i.e., on the way in which they are

TABLE 3

Estimates of the adaptive values of the karyotypes in the Misima experiments

Karyotype	Cont-I	Cont-II	Cont-III	DDT	DLR-I	DLR-II
ST/ST	0.69	1.13	1.11	0.97	1.48	1.22
ST/AR	1.00	1.00	1.00	1.00	1.00	1.00
ST/CH	0.80	0.77	0.88	0.44	1.29	0.16
ST/PP	0.72	0.82	0.88	0.97	1.18	1.09
AR/AR	0.54	0.63	0.65	0.34	1.14	0.39
AR/CH	0.19	0.77	0.32	0.68	0.62	0.79
AR/PP	0.31	1.08	0.47	0.20	0.75	0.87
CH/CH	0.54	0.79	0.52	0.63	0.14	0.81
CH/PP	0.47	0.12	0.59	0.40	0.63	1.07
PP/PP	0.59	0.15	0.78	0.45	0.86	0.91
Chi-squares						
ST	8.59	12.68	5.49	5.22	13.20	3.74
AR	19.29	27.14	13.25	21.17	30.60	24.19
CH	8.38	7.13	18.08	4.23	14.32	45.74
PP	11.60	6.43	14.05	29.37	14.48	13.48
Total	47.86	53.38	50.87	59.98	72.61	87.15
d.f.	18	15	15	18	15	15

TABLE 4

Estimates of the adaptive values of the karyotypes in the New York experiments

Karyotype	Cont-I	Cont-II	DDT-I	DDT-II	DLR-I	DLR-II
ST/ST	0.35	0.29	0.72	0.47	0.85	0.31
ST/AR	1.00	1.00	1.00	1.00	1.00	1.00
ST/CH	0.76	0.11	0.74	0.83	0.40	0.60
ST/PP	0.61	0.69	0.85	0.76	0.75	0.43
ST/TL	0.51	0.65	0.75	0.62	0.65	0.77
AR/AR	0.58	0.57	0.81	0.62	0.92	0.61
AR/CH	0.26	0.62	0.80	0.38	0.35	0.21
AR/PP	0.33	0.19	0.53	0.52	0.74	0.61
AR/TL	0.45	0.38	0.67	0.56	0.69	0.57
CH/CH	0.26	0.35	0.48	0.36	0.75	0.59
CH/PP	0.26	0.57	0.27	0.10	0.69	0.49
CH/TL	0.19	0.48	0.21	0.20	0.47	0.33
PP/PP	0.45	0.37	0.55	0.32	0.30	0.49
PP/TL	0.27	-0.02	0.20	0.17	0.39	0.18
TL/TL	0.35	0.26	0.33	0.36	0.10	0.02
Chi-squares						
ST	14.28	7.78	9.08	5.54	8.87	8.35
AR	8.96	3.07	4.20	6.62	9.58	7.70
CH	15.98	21.11	27.75	9.95	8.70	12.51
PP	12.80	7.80	11.81	2.50	16.56	13.30
TL	27.76	26.17	18.27	5.48	5.79	12.96
Total	79.77	65.94	71.12	30.09	49.51	54.82
d.f.	22	22	22	22	22	22

correlated. The estimates of the adaptive values are consistently highly correlated, one with another. These correlations make comparisons of different populations meaningful. Analysis of populations with only two or three kinds of chromosomes shows that the variability decreases rapidly as the number of kinds of chromosomes becomes smaller (DuMOUCHEL and ANDERSON, *loc. cit.*)

A comment regarding our model is in order. Adaptive values of a genotype or karyotype may not be constant, but may change as the physical and biotic environments are altered. Such changes would be difficult to specify mathematically, and the model was chosen as one that is practically utilizable. The adaptive values estimated should give at least good first approximations to the true values.

Estimates of the Adaptive Values: In Tables 3 and 4 the estimates of the adaptive values are given in relation to that of the ST/AR karyotype which is taken to have the value of unity. In the experimental populations raised in New York, ST/AR is invariably the fittest karyotype, but those raised in Japan are much more variable, and the ST/ST has an estimated fitness greater than unity in most populations. Despite the high variability, the heterokaryotypes give rather consistently higher adaptive values than the homokaryotypes. The Misima and the New York experiments differ, however, in that the ST/ST homokaryotype is fitter than AR/AR in Misima, while AR/AR is consistently superior to ST/ST in New York. The homokaryotypes CH/CH, PP/PP and TL/TL have usually low adaptive values.

The chi-squares show that in a majority of instances the estimates of the adaptive values obtained do not give a really satisfactory fit to the changes in the frequencies of the different gene arrangements observed in the different generations of the experiments. In the New York experiments the CH and TL arrangements give generally the worst fit. It is probably these gene arrangements that change their relative fitness depending on the relative frequencies of the other chromosomal types in the populations. Such changes are strongly suggested by the data of PAVLOVSKY and DOBZHANSKY (1966) for the gene arrangements in *D. pseudoobscura*; an elegant demonstration of such frequency-dependent selection has also been given for an enzyme polymorphism in *D. melanogaster* by KOJIMA and YARBROUGH (1967).

The sets of the estimates of the adaptive values in different populations may now be compared. The chi-squares calculated for this purpose are listed in Table 5. It should be noted that the replicate populations, both those treated with insecticides and the controls, gave concordant results in New York, but discordant ones in two of the four comparisons in the Japanese data. This is not really unexpected, because one of the controls and one of the dieldrin-treated populations were studied in Misima at a different time than their replicates (see above).

Greater Doses of the Insecticides: In the American experiments described above the insecticide treatments were deliberately made very weak since, at least outside the agricultural zones, natural populations of *D. pseudoobscura* are rarely if ever exposed to more than traces of the insecticide contaminants. The experimental treatments caused at most a 10 percent mortality of the treated flies, and in many

TABLE 5

Comparison of the sets of the adaptive values obtained for different populations. J—experiments made in Japan, A—made in New York. Con, DDT, and DLR—Control, DDT-treated and Dieldrin-treated populations respectively

Comparison		Chi-square	d.f.	Probability = P
Replicates	J-Con-1 vs. J-Con 2	14.64	9	$0.10 < P < 0.20$
	J-Con-1 vs. J-Con 3	25.65	9	$0.001 < P < 0.01$
	J-Con-2 vs. J-Con 3	7.46	9	$0.50 < P < 0.70$
	J-DLR-1 vs. J-DLR-2	17.88	9	$0.02 < P < 0.05$
	A-Con-1 vs. A-Con 2	1.11	14	> 0.90
	A-DDT-1 vs. A-DDT-2	3.26	14	> 0.90
Controls-Treated	A-DLR-1 vs. A-DLR-2	6.16	14	> 0.90
	J-Con-1 vs. J-DDT	18.98	9	$0.02 < P < 0.05$
	J-Con-2 vs. J-DLR-1	30.29	9	< 0.001
	J-Con-3 vs. J-DLR-2	1.73	9	> 0.90
	A-Con-1 vs. A-DDT-1	4.62	14	> 0.90
	A-Con-2 vs. A-DDT-2	5.05	14	> 0.90
	A-Con-1 vs. A-DLR-1	7.37	14	> 0.90
	A-Con-2 vs. A-DLR-2	10.89	14	$0.50 < P < 0.70$

cases they caused no perceptible mortality at all. Their influence on the selection could nevertheless be considerable, via a differential fecundity rather than the immediate mortality of the treated flies. The negative outcome of the experiments induced us to try stronger treatments. Six new populations were set up exactly as the six in the original New York series of experiments. The flies were exposed, however, to treatments of 5 hours on papers with 1 percent DDT, or 5 hours on

TABLE 6

Frequencies, in percent, of five kinds of chromosomes in populations surviving strong insecticide treatments

Chromosomes	Control No. 236			Control No. 241		DDT No. 237	
	S-0	S-1	S-2	S-1	S-2	S-1	S-2
ST	17.5	26.3	42.0	31.0	32.3	26.3	40.3
AR	17.5	23.3	26.7	23.3	25.3	15.3	18.0
CH	17.5	13.3	4.3	15.3	17.0	15.0	12.3
PP	25.0	18.0	12.7	16.0	11.0	22.7	14.7
TL	22.5	19.0	14.3	14.3	14.3	20.7	14.7
		DDT No. 239		Dieldrin No. 238		Dieldrin No. 240	
		S-1	S-2	S-1	S-2	S-1	S-2
ST		28.7	53.3	32.0	35.3	27.7	40.3
AR		18.3	14.0	19.7	17.0	24.3	21.3
CH		18.0	11.0	14.3	16.7	16.7	13.0
PP		19.7	11.0	19.0	16.7	17.0	11.7
TL		15.3	10.7	15.0	14.3	14.3	13.7

Homogeneity Chi-Squares on Data of Table 6

Comparison	Type	Chi-square*	Probability
236 × 241	Con × Con	30.93	<0.005
237 × 239	DDT × DDT	15.61	0.025–0.05
238 × 240	DLR × DLR	9.81	0.25–0.5
241 × 239	Con × DDT	34.59	<0.005
241 × 237	Con × DDT	23.34	<0.005
236 × 237	Con × DDT	24.36	<0.005
241 × 240	Con × DLR	6.33	0.5–0.75
236 × 238	Con × DLR	39.97	<0.005

* 8 degrees of freedom.

papers impregnated with 0.05 percent dieldrin. This treatment results in a mortality exceeding 50 percent, and usually close to 90 percent.

The experiment was continued for only two generations. The frequencies of the chromosomes with different gene arrangements observed in the populations are shown in Table 6. As expected, the frequency of ST chromosomes, and usually also of AR chromosomes, rose and those of PP, TL and usually CH fell. The matter of interest is, however, whether the changes in the treated populations were different from the controls and from each other. A way of testing for a differential selection of the inversions under heavy dosages of the insecticides is by chi-squares of homogeneity on the numbers of each karyotype. Pairs of populations were compared. The difference between the two controls is as large as the difference between untreated and insecticide-treated populations. The differences between the replicate populations treated with DDT or DLR are smaller. We conclude that the heavy insecticide treatment did not differentially select among the chromosomal types, since the two untreated populations differed about as much as any one untreated population did from any one of the treated populations.

DISCUSSION

The original purpose of the experiments reported in the present article was to test the hypothesis that the genetic changes observed in natural populations of *Drosophila pseudoobscura* may have been caused by contamination of the environments of the flies by traces of insecticides. The outcome of the experiments is in this respect, negative. We found no evidence that the natural selection processes in the populations treated with insecticides are meaningfully different from the untreated controls. In fact, the replicate control populations may diverge from one another as much as do the treated from the controls. The experiments did, however, produce new evidence of the delicate sensitivity of the adaptive values of karyotypes to even slight environmental differences. The experimental populations studied in Japan were descended from the same wild progenitors as those studied in New York; the former populations had four kinds of third chromosomes, while those in New York had five. Chromosomes with ST and AR gene arrangements were, however, included in all populations; in Japan the Standard

(ST) homokaryotype was mostly superior in fitness to the Standard/Arrowhead heterokaryotype; in New York ST/AR had invariably higher adaptive values than ST/ST. As a result, the Japanese populations tended to become monomorphic for ST, while in New York equilibria were established with frequencies of AR usually higher than those of ST. Just what environmental variable was responsible for this striking difference is unknown.

The causes responsible for the genetic alterations in natural populations, chiefly in those of the Pacific Coast states, remain unascertained; one possibility after the other has been ruled out (DOBZHANSKY, ANDERSON and PAVLOVSKY 1966, and references therein), and now the insecticide hypothesis must seemingly be discarded. This makes it necessary to entertain even more seriously than before the "genetic" hypothesis: the emergence, by mutation or by recombination, of new gene complexes locked up in the inversions (DOBZHANSKY 1963). The appearance, for example, of a ST third chromosome more fit than the old one in the homokaryotype and, for example, in the Standard/Pikes Peak heterokaryotype, would cause increases in the populations of the frequencies of ST and PP, and decreases of other chromosomal types. Indications of changes in the adaptive values of certain karyotypes with time have, indeed, been obtained (PAVLOVSKY and DOBZHANSKY 1966). A definitive proof is, of course, difficult to obtain, since chromosomes taken from natural populations years or decades ago and kept since in laboratory cultures may undergo changes in the laboratory as well as in nature.

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SUMMARY

Experimental populations of *Drosophila pseudoobscura*, polymorphic for four or for five gene arrangements in their third chromosomes, were treated with weak doses of DDT and of dieldrin. The adaptive values of the karyotypes were estimated from the changes in their frequencies observed with time in the populations. No meaningful differences between the insecticide-treated and control populations were observed. The sets of the adaptive values proved, however, to be appreciably different in populations studied in Japan from those studied in New York, thus giving new evidence of the sensitivity of these adaptive values to even slight environmental variations.

LITERATURE CITED

- CAVALLI, L. L., 1950 Recent applications of biometrical methods in genetics. (2). The analysis of selection curves. *Biometrics* 6: 208-220.
- DOBZHANSKY, TH., 1963 Genetics of natural populations. XXXIII. A progress report on genetic

- changes in populations of *Drosophila pseudoobscura* and *Drosophila persimilis* in a locality in California. *Evolution* **17**: 333–339.
- DOBZHANSKY, TH., W. W. ANDERSON, and O. PAVLOVSKY, 1966 Genetics of natural populations. XXXVIII. Continuity and change in populations of *Drosophila pseudoobscura* in western United States. *Evolution* **20**: 418–427.
- DOBZHANSKY, TH., W. W. ANDERSON, O. PAVLOVSKY, B. SPASSKY, and C. J. WILLS, 1964 Genetics of natural populations. XXXV. A progress report on genetic changes in populations of *Drosophila pseudoobscura* in the American Southwest. *Evolution* **18**: 164–176.
- DUMOUCHEL, W., and W. W. ANDERSON, 1968 Analysis of selection in experimental populations. *Genetics* **58**: 435–449.
- KOJIMA, K., and K. M. YARBROUGH, 1967 Frequency-dependent selection at the esterase-6 locus in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **57**: 645–649.
- LEVENE, H., O. PAVLOVSKY, and TH. DOBZHANSKY, 1954 Interaction of the adaptive values in polymorphic experimental populations of *Drosophila pseudoobscura*. *Evolution* **8**: 335–349.
- OSHIMA, C., and T. WATANABE, 1965 The effect of insecticide selection on experimental populations of *Drosophila pseudoobscura*. *Drosophila Inform. Serv.* **41**: 140–141. ——— 1966 The effect of insecticide selection in experimental populations of *D. pseudoobscura*. *Ann. Report Natl. Inst. Genet. Japan* No. 16: 19–20.
- PAVLOVSKY, O., and TH. DOBZHANSKY, 1966 Genetics of natural populations. XXXVII. The co-adapted system of chromosomal variants in a population of *Drosophila pseudoobscura*. *Genetics* **53**: 843–854.
- WRIGHT, S., and TH. DOBZHANSKY, 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. *Genetics* **31**: 125–156.