GENETICS OF NATURAL POPULATIONS. XXXVII. THE COADAPTED SYSTEM OF CHROMOSOMAL VARIANTS IN A POPULATION OF *DROSOPHILA PSEUDOOBSCURA**

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NATURAL populations of many species of Drosophila are polymorphic with respect to inversions of blocks of genes in their chromosomes. DOBZHANSKY (1943) showed that the relative frequencies of the chromosomal polymorphs undergo cyclic seasonal changes in certain populations of *Drosophila pseudoobscura* in California. This made it probable that the polymorphism is maintained by natural selection. WRIGHT and DOBZHANSKY (1946) reproduced some of the natural frequency changes in experimental populations kept in the laboratory in population cages. The experimental results were consistent with the view that the heterokaryotypes (i.e., individuals with two chromosomes of a pair having different gene arrangements) were heterotic, and had adaptive values superior to the homokaryotypes (the two chromosomes of a pair with similar gene arrangements). The quantitative estimates of the adaptive values showed that the heterokaryotypes had in some cases more than double the fitness of the homokaryotypes. The picture became more complex with the finding that the adaptive values (= Darwinian fitnesses) of the karyotypes were highly sensitive to environmental variables, and that cytologically identical chromosomes of different geographic origins may have different effects on fitness (DOBZHANSKY 1948, 1957). Clearly, the fitness is not an intrinsic property of the gene arrangement in the chromosome, it depends also on the polygene complex this chromosome carries. LEVENE, PAVLOVSKY and DOBZHANSKY (1954, 1958) showed further that the adaptive values of the karyotypes may depend upon their relative frequencies and upon the presence of other karyotypes in the same environment. Thus, the relative fitness of karyotypes A and B may change when karyotypes C and D are introduced in the population. Some natural populations contain several (up to eight) chromosomal variants, the combinations of which give rise to an array of homo and heterokaryotypes. The relative fitnesses within this array depend upon the coadaptation of the gene complexes (= supergenes) in the respective chromosomes.

The purpose of the present work is to explore the system of coadaptive relationships between the chromosomes found in the natural population of a single locality—Mather, in the Sierra Nevada of California. This particular population has added interest because its genetic composition underwent pronounced changes

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during the period it was under observation (1945 to 1965; see DOBZHANSKY 1964 and unpublished data). The coadapted system is evidently subject to changes rapid enough to be observed within an investigator's lifetime.

MATERIALS AND METHODS

The early experiments with chromosomes from Mather (DOBZHANSKY 1948) were made with materials collected in 1945 and 1946. For the present experiments we have used the descendants of the wild progenitors collected in June and July, 1959. Because the Mather population has changed genetically between 1945 and 1959 (DOBZHANSKY 1963), the "new" chromosomes were probably not identical in gene contents with those experimented with earlier.

The progenies of wild-collected flies were put through a series of crosses designed to obtain a collection of strains homozygous for different gene arrangements in the third chromosomes. For this purpose, we have used an "analyzer strain," which carried the dominant mutants Blade (*Bl*) and Scute (*Sc*) (Standard gene arrangement) in one of its third chromosomes, and Lobe (*L*) (Santa Cruz gene arrangement) in the other third chromosome. Wild-collected males, or single sons of wild-collected females, were crossed in individual cultures to *Bl Sc/L* females. Seven to ten larvae from each progeny were examined cytologically in salivary-gland preparations. If they contained desired kinds of chromosomes, several F_1 males (usually five) were backcrossed individually to *Bl Sc/L* females from the stock culture. The salivary-gland chromosomes were again examined in the progenies, to identify the cultures which had the desired gene arrangements. *Bl Sc* females and males (or *L* females and males, depending upon which gene arrangement was being dealt with) were selected and inbred. In the next generation, there appear wild-type and *Bl Sc* (or *L*) flies; the former contain the desired chromosome in double dose. About a dozen strains homokaryotypic for each of the following gene arrangements were obtained: Standard (ST), Arrowhead (AR), Chiricahua (CH), Pikes Peak (PP), and Tree Line (TL). For descriptions of these chromosomes, see DOBZHANSKY 1944. It should be noted that the method used tends to eliminate the "wild" chromosomes which act as lethals, semilethals, and sterility inducers in double dose. This elimination is particularly likely to occur in the rarer gene arrangements (CH, PP, TL), while lethals may be retained in the common ones (ST and AR).

The experimental populations were maintained in wood and glass population cages of the type described previously (WRIGHT and DOBZHANSKY 1946 and elsewhere). To obtain a population with initial frequencies of 50% of each of two gene arrangements, females of all available strains of one of them (say, ST) were crossed in ordinary culture bottles to males of all strains of the other (say, AR). The reciprocal cross was also made. The F_1 flies are inversion and genic heterozygotes, and are expected to have hybrid vigor. About 2000 of these flies were introduced in a population cage to become founders of the experimental population. Populations with three or four kinds of chromosomes were made using F_1 hybrids between all the strains with desired gene arrangements. A complication arises if the desired initial frequencies of the gene arrangements should be uneven, say 75% of one and 25% of another. Some of the founders are then homokaryotypic; to give them as much hybrid vigor as possible, the founding flies are obtained by intercrossing all the available strains with a given gene arrangement.

Unless otherwise specified, all experimental populations were kept at 25°C, until May of 1962 in incubators with forced air circulation and thereafter in a constant temperature room. The chromosome samples consisted of 300 chromosomes, scored in 150 larvae, taken in six subsamples, usually on six successive days. The sample data is the mean day on which the subsamples were taken. All the chromosomes were diagnosed by one of us (Th. D.).

Estimation of the adaptive values: The experimental data consist of the karyotype frequencies in the populations at the times when the samples were taken. WRIGHT (in WRIGHT and DOBZHANSKY 1946) devised a method of least squares for estimation of the adaptive values of the karyotypes; the estimates arrived at are those which minimize the sum of squared differences between the observed and the expected values in the chromosome frequencies. We are greatly obligated to PROFESSOR WRIGHT for making a most searching and critical analysis of the new data,

which has unfortunately shown that the method used in 1946 fails to give satisfactory results when applied to some of the present data (private communication). One of the assumptions on which the method is based is that the adaptive values of the karyotypes are constant over the frequency range observed in the experiments. There is considerable evidence that in the new data this is not always the case. When the differences in the karyotype frequencies in the successive samples (Δq) are plotted against these frequencies (q), only the middle portions of the resulting plots can be fitted by linear regression lines.

LEVENE (in DOBZHANSKY and LEVENE 1951) and PROUT (1966) developed an alternative method, whereby the adaptive values are estimated on the basis of three reasonably well spaced karyotype frequency observations. If these observations are not too far apart in time, the assumption that the adaptive values remain approximately constant is rendered plausible. In principle, this method can be used to detect changes in the adaptive values when a given karyotype is frequent and when it is rare. The difficulty is that the sampling errors of the individual frequency observations (in our experiments based on scoring of 300 chromosomes) make the results fluctuate widely. This becomes especially serious when the frequencies approach asymptotically their equilibrium levels, and the Δq values become of the same order as their sampling errors. These considerations have forced us to refrain from making precise estimates of the adaptive values. Fortunately, certain meaningful conclusions can be drawn from comparisons of different populations, especially of those started with founders remote from the equilibrium frequencies.

RESULTS

Bichromosomal populations: The starting dates and the kinds of the chromosomes present in the experimental populations are listed in Table 1. Among the 25 populations studied, 21 were bichromosomal, i.e., had two kinds of chromosomes, and 4 were multichromosomal, with three or four kinds of chromosomes. The percentage frequencies of the different gene arrangements observed in the bichromosomal populations are reported in Tables 2a and 2b. The frequency of only one gene arrangement is given, the other being obviously 100 minus the frequency of the first.

The most common gene arrangements in the population of Mather are ST and

TABLE 1

A review of the populations raised

No.	Chromosomes	Started	No.	Chromosomes	Started
183	AR + PP	February 9, 1960	217	ST + AR	April 27, 1961
184	AR + PP	February 11, 1960	218	PP + CH	June 6, 1961
185	PP + TL	February 16, 1960	219	ST + AR + CH + PP	February 20, 1962
186	ST + AR	February 22, 1960	220	ST + AR + CH + PP	February 20, 1962
187	ST + PP	March 2, 1960	221	ST + TL	February 18, 1964
189	ST + PP	March 5, 1960	222	ST + AR	February 18, 1964
202	AR + CH	November 21, 1960	223	ST + AR	February 18, 1964
203	PP + CH	November 25, 1960	224	AR + TL	February 18, 1964
204	AR + PP	December 16, 1960	225	ST + AR	March 17, 1964
207	AR + PP	December 23, 1960	226	ST + AR	March 17, 1964
210	ST + CH	January 5, 1961	227	PP + TL	April 22, 1964
214	PP + TL	April 19, 1961			
215	ST + AR + PP	April 19, 1961			
216	ST + AR + PP	April 22, 1961			

TABLE 2a

Chromosome frequencies observed in populations with two kinds of chromosomes

No.	Chromosomes	Percentage	0 Days	35 Days	70 Days	105 Days	140 Days	210 Days	225 Days	280 Days	300 Days	365 Days	455 Days
183	AR + PP	% AR	50.0	57.0	75.3	83.7	89.0	...	86.3	...	85.3	85.7	91.7
184	AR + PP	% AR	50.0	59.0	75.0	74.7	77.0	...	83.3	...	87.3	88.0	91.7
204	AR + PP	% AR	50.0	59.7	68.3	74.7	81.0	82.0	...	89.3	...	90.0	93.7†
207	AR + PP	% AR	50.0	59.0	69.3	75.7	77.3	86.7	...	86.3	...	91.0	90.0‡
187	ST + PP	% ST	50.0	64.3	62.0	73.3	76.0	...	85.7	...	85.3	88.7	...
189	ST + PP	% ST	50.0	59.7	69.7	67.0	78.3	...	86.7	...	87.7	86.0*	...
185	TL + PP	% PP	50.0	47.7	45.7	39.0	39.0	...	46.0	...	42.7	46.3	...
214	TL + PP	% PP	75.0	63.7	55.7	55.7	55.3	...	50.7	...	...	...	...
227	TL + PP	% PP	75.0	67.0	58.3	...	...	40.3	...	39.0	...	41.0	...
203	CH + PP	% PP	50.0	51.0	45.3	45.7	46.0	...	...	...	...	...	...
218	CH + PP	% PP	80.0	64.3	61.7	54.7	51.3	54.0	...	45.3	42.3	44.7	...
210	ST + CH	% ST	50.0	63.7	66.7	70.3	75.7	...	75.7	...	89.0	87.7	84.7
221	ST + TL	% ST	50.0	61.3	72.0	66.0	71.3	84.7	...	89.0	...	86.7	92.3
202	AR + CH	% AR	50.0	61.0	66.7	65.3	73.7	...	81.0	...	86.0	90.3	96.0
224	AR + TL	% AR	50.0	59.3	63.0	84.3	85.0	84.3	...	91.3	...	92.0	94.0
186	AR + ST	% ST	50.0	49.0	47.3	52.0	52.7	...	52.7	...	55.0	59.3	...
217	AR + ST	% ST	25.0	31.3	34.3	37.3	25.7	...	25.3	...	27.3	28.3	27.7
222	AR + ST	% ST	75.0	68.7	72.0	...	...	...	...	...	...	...	...
223	AR + ST	% ST	75.0	69.7	69.3	...	...	...	...	...	...	...	...
225	AR + ST	% ST	25.0	28.7	29.3	30.0	...	...	...	...	...	...	...
226	AR + ST	% ST	25.0	32.0	36.3	31.0	...	...	...	...	...	...	...

* 372 Days † 427 Days ‡ 421 Days

TABLE 2b

Chromosome frequencies observed in populations with two kinds of chromosomes

No. 183 AR+PP percent AR		No. 204 AR+PP percent AR		No. 207 AR+PP percent AR		No. 217 AR+ST percent ST		No. 210 ST+CH percent ST	
Days	Percent	Days	Percent	Days	Percent	Days	Percent	Days	Percent
572	96.0	546	94.0	545	93.3	545	29.7	545	85.7
730	95.3	635	95.7	635	91.7	649	26.0	649	84.7
845	100.0	725	96.3	720	97.3	725	28.0	...	...
...	...	815	96.3	815	98.0	813	26.0	...	...
...	...	...	...	...	...	905	34.3	...	...
...	...	...	...	...	...	1001	31.0	...	...

AR; between 1946 and 1954 AR was the most frequent, while from 1957 to 1965 ST became the prevalent one. Six experimental populations, Nos. 186, 217, 222, 223, 225, and 226 had ST and AR chromosomes. No. 186 was started earliest, with ST and AR at 50 percent. The frequency of ST rose slowly, reaching 59% a year from the start. So slight a frequency change led us to suspect that the equilibrium value for the ST and AR polymorphs may lie in the vicinity of 60% ST. To test this hypothesis, populations Nos. 222 and 223 were started with 75%

ST and 25 AR, while in populations Nos. 217, 225 and 226 the initial frequencies were reversed. None of these populations gave changes indicating convergence towards fixed equilibrium values. No. 217 was maintained for almost three years, and it fluctuated irregularly between the starting frequency and 37% of ST. In Nos. 222 and 223 ST decreased, and in Nos. 225 and 226 it increased slightly in two to three generations.

These results are best interpreted as meaning that at 25°C the three karyotypes, ST/ST, ST/AR, and AR/AR are not appreciably different in fitness. The heterokaryotype may be only slightly heterotic, and the relative fitnesses of the homokaryotypes appear to be sensitive to environmental fluctuations which unavoidably occur in experimental populations maintained for many months. An attempt was made to change the situation by altering the environment deliberately. After the populations Nos. 222, 223, 225, and 226 were kept for two or three generations at 25°, they were transferred in a constant temperature room at 16°. The results are shown in Table 5. The chromosome frequencies scarcely changed in Nos. 222 and 223 in which ST had a frequency close to 70% at the start, while in Nos. 225 and 226 ST rose consistently and significantly from 30 or 31% at the start to about 50% a year later. It appears, then, that at 16° the homokaryotype ST/ST has a higher fitness than AR/AR.

This behavior of ST and AR chromosomes is rather different from that observed in the experiments conducted in 1945-1947 with ST and AR chromosomes collected at Mather in 1945 (DOBZHANSKY 1948). A population was started with about 32% ST and 68% AR, changed at 25° to 60 and 40% of ST and AR respectively within about 3½ months, remained in that state for 3 more months, was transferred to 16° and did not change further for about 6 months. Its behavior at 25° led to the estimates of the fitness of the three karyotypes as follows:

ST/ST	ST/AR	AR/AR
0.79	1	0.58

The population of the Mather locality was, as pointed out above, changing genetically between 1945 and 1959, when the "old" and the "new" chromosomes were taken. It is quite probable that the adaptive values of the karyotypes observed in the old and in the new experiments are really different, as the experimental results indicate. On the other hand, the sensitivity of these values to environmental changes makes this conclusion not quite compelling.

The experimental populations involving PP chromosomes are most interesting, because this gene arrangement was rare or absent in California before 1946 but increased in frequency thereafter (DOBZHANSKY 1963; DOBZHANSKY, ANDERSON, PAVLOVSKY, SPASSKY and WILLS 1964). The old experiments did not include this gene arrangement. Populations 183, 184, 204, and 207 were all started with 50% of PP and AR chromosomes (Tables 2a and 2b). After 845 days, PP has disappeared in one of the populations and become rare in the others. The changes observed in the four populations are not quite consistent, since No. 183 had higher frequencies of AR chromosomes between the 70th and the 140th days than did the other populations. However, the populations eventually converged; the loss

of PP in No. 183 and its retention at very low frequencies in the other populations was probably accidental. Appreciable changes in chromosome frequencies between consecutive samples were taking place for less than a year; pooling the data for the four populations until the 225th day, and using Wright's least square method of estimation, we obtain the following adaptive values:

AR/AR	AR/PP	PP/PP
1.07	1	0.39

The heterokaryotype is assigned the adaptive value of unity arbitrarily; the fitness of AR/AR is as high or higher as that of the heterokaryotype, while that of PP/PP is very low. PP chromosomes thus are not likely to be retained indefinitely in a population with AR, kept at the environmental conditions of our experiments.

PP chromosomes were tested together with ST in populations Nos. 187 and 189. ST being the commonest chromosome in the Mather locality, its behavior with PP is particularly important for the maintenance of the latter. In both populations equilibria seem to have been reached at frequencies of between 85 and 90% ST. This indicates that the ST/PP heterokaryotype is heterotic. An attempt to estimate the fitnesses by the Wright method during the period between 0 and 225 days gives the following result:

ST/ST	ST/PP	PP/PP
0.98	1	0.39

Since both homokaryotypes are lower in fitness than the heterokaryotype, PP chromosomes can be held in balanced equilibrium in populations with ST. However, the estimates obtained would predict an equilibrium at only about 3% of PP chromosomes $0.02/(0.02 + 0.61)$, whereas the populations seemed to have reached an equilibrium with 10 to 15% PP (Table 2). This may have been due either to an uncontrolled environmental change, or to PP/PP homokaryotypes increasing in relative fitness when their frequency in the populations become low.

The combinations of PP with TL and CH are of less momentum for an elucidation of the situation in nature, because PP/TL and CH/PP heterokaryotypes are rarely formed on account of these gene arrangements being infrequent in the Mather locality. Nos. 185, 214 and 227 combining PP and TL gave inconsistent results. Starting with 50% of each of the two chromosomes, PP seemed to decrease slightly in No. 185 and to increase in No. 214. In No. 227, PP declined from 75% at the beginning to about 40% at 210 days, and remained at this frequency until the population was discarded when it was one year old. No significant change was observed in No. 203, where CH and PP were started with equal frequencies; in No. 218 the frequency of PP fell from 80 to what looked like an equilibrium at about 45%. This seems to indicate a sequence of the fitnesses CH/PP>CH/CH>PP/PP.

CH and ST chromosomes were tested together in population No. 210, and CH and AR in No. 210 (Tables 2a and 2b). Unfortunately no replications were made. The changes in No. 210 were so erratic that they cannot be fitted to a set of adaptive values. However, in about a year from the start, this population apparently

reached an equilibrium at about 85 to 90% ST, and conserved it until the population was terminated at the age of 649 days (Table 2b). The fitness sequence is, hence, ST/CH>ST/ST>CH/CH. This is the same sequence which was obtained with the old ST and CH chromosomes (DOBZHANSKY 1948). The combination of AR and CH chromosomes (population No. 202) gave during the first 225 days changes which can be fitted to adaptive values:

AR/AR	AR/CH	CH/CH
0.82	1	0.26

If the relative fitness remained unchanged at different frequencies of the karyotypes, an equilibrium would be expected with about 80% AR and 20% CH chromosomes. The frequency of AR stood, however, at 96% when the population was terminated at the age of 455 days. This is strikingly different from the result obtained in 1946 to 47 with the "old" AR and CH chromosomes from Mather, which reached an equilibrium between 50 and 60% AR (DOBZHANSKY 1948). An appreciable change in the properties of these chromosomes in the Mather population is indicated.

Changes are also indicated in the behavior of TL chromosomes with ST and AR. In the old experiments, TL was close to elimination in the population with ST, but seemed to have reached an equilibrium at 20 to 25% level with AR. Populations Nos. 221 and 224 with "new" TL, ST and AR chromosomes had less than 10% TL at 455 days. The early changes were, however, too erratic to warrant computation of the adaptive values.

Multichromosomal populations: Experimental bichromosomal populations, with only two kinds of gene arrangements, differ greatly from natural populations. The experimental populations contain fewer karyotypes and, especially if they are made with gene arrangements that are rare in nature, the karyotypes most frequent in the experimental populations occur in natural ones only as exceptions. We have studied four multichromosomal populations; Nos. 215 and 216 had the three gene arrangements ST, AR and PP, and Nos. 219 and 220 had four, ST, AR, PP and CH. The changing chromosome frequencies are recorded in Tables 3 and 4.

The estimation of the adaptive values in multichromosomal populations is beset with difficulties. LEVENE, PAVLOVSKY and DOBZHANSKY (1954, 1958) showed that the relative fitness of two karyotypes, A and B, may depend on the presence or absence of other karyotypes, say C and D, in the same medium. This means that the adaptive values of the karyotypes observed in bichromosomal populations may well be altered in the multichromosomal ones. The disturbing effects of the possible changes in the adaptive values with changing frequencies of the karyotypes may also be present. We are indebted to MR. W. W. ANDERSON for having made estimates of the adaptive values from the data on the tri- and tetrachromosomal populations (Nos. 215, 216, 219, and 220), using the CDC 160-A computer, kindly provided by PROFESSOR H. K. HARTLINE. A striking, and probably significant, feature of these estimates of the adaptive values in multichromosomal populations is that almost all the heterokaryotypes (with the apparent exception of

TABLE 3

Chromosome frequencies observed in populations with three kinds of chromosomes

	Population No. 215			Population No. 216		
Days	ST	AR	PP	ST	AR	PP
0	25.0	25.0	50.0	25.0	25.0	50.0
35	24.7	38.0	37.3	34.0	29.7	36.3
70	36.7	34.0	29.3	39.3	25.7	35.0
97	42.7	35.7	21.7	50.0	20.0	30.0
141	44.3	40.3	15.3	56.0	31.7	12.3
210	47.0	41.7	11.3	55.7	34.7	9.7
280	54.7	39.0	6.3	57.0	36.0	7.0
361	45.7	46.7	7.7	50.3	41.3	8.3
455	41.0	54.3	4.7	50.3	44.7	5.0
545	47.0	50.0	3.0	53.7	39.3	7.0
655	50.7	49.0	0.3	57.3	41.3	1.3

AR/PP in the population 220) are superior to all the homokaryotypes. This could not have been predicted from the results of the experiments with bichromosomal populations. In some of the latter no balanced equilibria were achieved, suggesting that some of the heterokaryotypes lacked heterosis (i.e., AR/AR is equal or superior to AR/PP in the bichromosomal but not in most multichromosomal populations).

Although the environments in the population cages are certainly not identical with those in which the flies live in nature, the relative fitness of the heterokaryotypes in the experiments agrees fairly well with what one could infer from their frequencies in nature when the progenitors of the experimental flies were collected. The frequencies in nature were (DOBZHANSKY 1963):

$$ST > AR > PP > CH$$

TABLE 4

Chromosome frequencies observed in populations with four kinds of chromosomes

	Population No. 219				Population No. 220			
Days	ST	AR	CH	PP	ST	AR	CH	PP
0	10.0	10.0	40.0	40.0	10.0	10.0	40.0	40.0
35	15.7	15.7	29.3	39.3	18.0	11.3	31.3	39.3
54	22.7	15.0	25.7	36.7	20.0	8.7	33.3	38.0
105	32.3	13.0	22.0	32.7	37.3	11.0	21.3	30.3
190	41.0	27.3	19.0	12.7	42.7	20.3	20.0	17.0
240	41.3	33.7	9.3	15.7	50.3	27.0	10.3	12.3
300	48.0	37.7	6.0	8.3	50.3	24.7	12.0	13.0
365	48.7	33.7	11.3	6.3	57.0	27.7	9.7	5.7
455	52.7	32.3	7.7	7.3	49.3	34.0	10.0	6.7
565	57.3	33.3	4.0	5.7	41.7	47.3	3.3	7.7
653	54.7	39.0	4.3	2.0	44.3	46.3	5.0	4.3

The adaptive values in the experimental populations with three gene arrangements are:

$$ST/AR > ST/PP > AR/PP > ST/ST > AR/AR > PP/PP$$

And in the populations with four gene arrangements the ordering of the adaptive values is:

$$ST/AR > CH/PP > ST/CH > ST/PP > AR/PP > AR/CH > ST/ST > AR/AR > PP/PP > CH/CH$$

In point of fact, the equilibria seemingly achieved in the populations 219 and 220 match fairly closely the frequencies of the different gene arrangements at Mather in 1957, 1959 and 1963, taking into account that the natural population had some gene arrangements not included in the experimental populations (DOBZHANSKY 1963; DOBZHANSKY *et al.* 1964).

As stated above, LEVENE, PAVLOVSKY and DOBZHANSKY observed appreciable changes in the adaptive values of certain karyotypes, depending upon the presence or absence of certain other karyotypes in the same populations. These experiments dealt with chromosomes of different geographic origin (southern California). The clearest case in the present data is that of ST and AR. All six bichromosomal populations with these two gene arrangements agree in showing that the heterokaryotype ST/AR is only slightly heterotic compared to ST/ST, and this latter is only mildly superior to AR/AR. However, in multichromosomal populations the heterokaryotype is far superior to the homokaryotypes. With all reasonable allowances for the imprecision of the estimates, this difference is too striking to be a matter of chance.

Fitness and environmental changes: Already WRIGHT and DOBZHANSKY (1946) found that the relative fitness of the karyotypes of *D. pseudoobscura* derived from the population of Mount San Jacinto, in southern California, was extremely sensitive to temperature. BEARDMORE and LEVINE (1963), and VAN VALEN, LEVINE and BEARDMORE (1963) studied this phenomenon further. SPIESS (1950) found a similar sensitivity in *D. persimilis*, but in this species the changes went, interestingly enough, in a direction opposite to that in *D. pseudoobscura*. The experimental populations described in the present article offered an opportunity to compare the behavior of the chromosomes of southern California origin with that of Mather chromosomes. With the former, the heterosis is strongly pronounced at 25°C, but the homo- and the heterokaryotypes are about equal at 16°. The populations 186, 217, 222, 223, 225, 226 (see above) showed little or no significant change in the frequencies of AR and ST chromosomes when kept at 25°, even though the initial frequencies of the gene arrangements in them varied from 25 to 75% ST. Populations Nos. 222, 223, 225 and 226 were transferred from the warm 25° room to that at 16°. The changes observed are shown in Table 5. The frequencies of ST rose in Nos. 225 and 226 in which they were initially low, but rose only slightly or not at all where they were in the neighborhood of 70%. The results observed in the populations 222 and 223 (Table 5) were quite different; with initial frequencies of about 70% ST, the trends were,

TABLE 5

Percentages of ST chromosomes in bichromosomal populations containing ST and AR gene arrangements and kept at 16°C

	Population			Population	
Days	222	223	Days	225	226
0*	72.0	69.3	0	30.0	31.0
35	71.7	68.0	70	40.0	37.5
70	73.7	75.3	140	45.0	41.7
140	71.7	75.3	210	49.7	46.3
210	73.3	75.0	280	43.0	44.0
...	...	...	380	53.0	48.0
...	...	...	450	51.0	53.0

* The entry at zero days is the frequency obtained at the time the populations were transferred from 25° to 16°.

if anything, towards slightly higher, not lower, frequencies. A tentative explanation that can be suggested is that the relative fitness of the karyotypes formed by the ST and AR chromosomes of Mather origin depends upon their frequencies in the populations, at least in the 16° environment.

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CONCLUSIONS AND SUMMARY

Experimental populations of *Drosophila pseudoobscura* were made in laboratory population cages, using various combinations of third chromosomes with different gene arrangements derived from the natural population of Mather, California. Complex coadaptive relationships between these chromosomes have been uncovered.

Bichromosomal experimental populations contain two kinds of chromosomes. The heterokaryotypes are, as a rule, superior in fitness to the homokaryotypes, and most populations attain equilibria at which both kinds of chromosomes are maintained at certain frequencies. Some combinations of chromosomes are, however, exceptions to the rule. They fail to produce heterotic heterokaryotypes and to establish balanced equilibria. Thus, the chromosomes with the PP gene arrangement are heterotic with ST but not with AR chromosomes. This incomplete coadaptation may be related to the fact that before 1946 PP chromosomes were rare or absent in most of California, and have spread in the late 1940's and 50's. The heterokaryotypes formed by chromosomes which are uncommon in the ancestral natural population are nevertheless mostly heterotic.

Multichromosomal populations contain three or four kinds of chromosomes with different gene arrangements. The adaptive values of all heterokaryotypes

in such populations turn out to be, with one possible exception, higher than the adaptive values of any of the homokaryotypes.

The relative adaptive values of certain karyotypes in bichromosomal populations differ appreciably from those of the same karyotypes in multichromosomal ones. Despite the low precision of the measurement of the adaptive values in our experiments, some of the differences seem to be real, and even qualitative rather than quantitative. LEVENE, PAVLOVSKY and DOBZHANSKY (1954, 1958), working with chromosomes of different geographic origin, observed similar situations: the fitness of a karyotype A in relation to B may depend upon the presence or absence of a karyotype C in the same population. There are some indications that the fitness of a karyotype may change as it grows or decreases in frequency in the population.

The experiments described in the present article have been made with chromosomes derived from population samples collected at Mather in 1959. Some similar experiments were made with chromosomes collected in the same locality in 1945 and 1946 (described in DOBZHANSKY 1948). The outcomes with the old and the new experiments with cytologically identical chromosomes are in some cases appreciably different. Making due allowances for the possible environmental disparities between the old and the new experiments, some of the differences observed are not accidental. They reflect the genetic reconstructions which have taken place in the natural populations of Mather, and in fact everywhere in California and in parts of adjacent states (DOBZHANSKY, ANDERSON, PAVLOVSKY, SPASSKY and WILLS 1964). The causes which have brought about these evolutionary events remain, however, unknown.

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